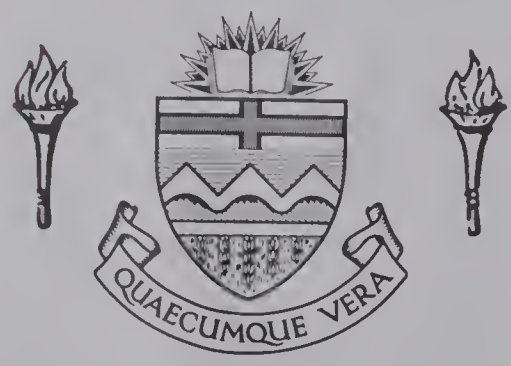


The University of Alberta
Printing Department
Edmonton, Alberta

THE UNIVERSITY OF ALBERTA

THE ULTRASTRUCTURE OF GERMINATING MARCROCONIDIA OF *NEUROSPORA CRASSA*

By

© FRANK KOZAR

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

FALL, 1969

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled The Ultrastructure of Germinating Macroconidia of Neurospora crassa submitted by Frank Kozar in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

To my wife Dolores Joan and our children

Arlene Ann and Roy Francis

ABSTRACT

The present paper describes further observations on the fine structure of germinating, as well as, resting conidia of Neurospora crassa. A comparison is made of single fixation in $KMnO_4$ with double fixation in glutaraldehyde and OsO_4. Special attention is given to the search for cellular organelles. Although endoplasmic reticulum and mitochondria are present in the ungerminated conidia, these organelles increase greatly during germination. Numerous osmiophilic bodies and vacuoles also appeared and as germination continued, these organelles increased in size and numbers. Numerous lomasomes, vesicular bodies and complex concentric membranes (mesosomes) associated with the cell membrane or occurring in the cytoplasm were also observed and described.

A number of other cellular organelles, among them microtubules, spherosomes, lipoproteinic sheets and crystalline inclusions are also reported. These are identified on the basis of morphological analogy with similar structures in other organisms.

ACKNOWLEDGEMENTS

The writer wishes to record his indebtedness to Dr. J. Weijer, Chairman, Department of Genetics, University of Alberta for introducing him to this field of research and for his constant guidance and valuable suggestions throughout the study.

Appreciation is also given to Dr. S.K. Malhotra, Electron Microscope Laboratory, Biological Sciences, University of Alberta for making available the electron microscope and for his help in evaluation and interpretation of some of the electron micrographs.

Gratitude is expressed to Dr. T. Shnitka for his unstinted help in evaluation and interpretation of some of the electron micrographs.

The technical assistance during all phases of the study provided by Mrs. Sita Prasad is gratefully acknowledged.

In conclusion the writer wishes to take this opportunity to thank the staff members of various departments both academic and technical, as well as all the graduate students for their assistance and for making my stay at this University a memorable one.

TABLE OF CONTENTS

TABLE OF FIGURES

FIGURE

FIGURE

FIGURE

INTRODUCTION

This thesis describes observations of the fine structures of germinating conidia of N. crassa. Special emphasis has been placed on the search for vesicular bodies and other cell organelles e.g., mesosomes, lomasomes and crystals in the resting, the growing, as well as the maturing conidium.

Although the formation and germination of endospores of bacteria have been extensively studied, it is only during the past decade that the ultrastructure of fungal conidia has been investigated. Shatkin (1959) and Shatkin and Tatum (1959) are early references in this area of investigation. In their ultrastructural study of N. crassa hyphae, they reported a chitinous polysaccharide wall, a sinuate plasma membrane adjacent to the inner wall often closely associated with the endoplasmic reticulum (ER), classical-type mitochondria, dense cytoplasmic particles and a double-membrane-bound nuclear region containing a nucleus.

Zalokar (1961), in a study comparing normal with centrifuged hyphae of Neurospora identified nuclei with nucleoli, mitochondria, endoplasmic reticulum, ribosomes, glycogen, fat bodies, vacuoles and vesicles which contained an inner canicular system of unknown nature. This latter investigation indicated that Neurospora hyphae do not have an extensive lamellar system.

Weiz (1965) pioneered an ultrastructural study correlating biochemical results with morphological changes in N. crassa conidia and reported that no significant difference in the ultrastructure of germlings could be detected when compared with ungerminated conidia.

She noted, however, the occurrence of vacuoles and large accumulations of lipid material in the conidia. Moreover, no change in the appearance of mitochondria was evident in the development of a germinating conidium. This latter observation, however, was challenged by Manocha (1968), who found that morphological changes occurred in the mitochondria during conidial germination. These changes in the morphology of the conidium were attributed to the transition from the dormant to the actively metabolizing vegetative state of the fungus.

Namboodiri (1966) was one of the first to investigate extensively the ultrastructure of conidia of N. crassa. He reported that the endoplasmic reticulum in conidia was sparse and discontinuous whereas in the mycelia it was well developed, not unlike that observed in the higher plants. The nuclei of conidia showed a double-membraned envelope with well defined pores. Continuities between the endoplasmic reticulum and the nuclear envelope were found to exist. Internuclear connections, as reported in the conidium of Aspergillus (Tanaka and Yanagita, 1963), were also prevalent in conidia.

Mitochondria showing variation in form and structure as well as vacuoles, with our without inclusions, were observed.

Instead of the fibriform network shown by Shatkin and Tatum (loc. cit.), Namboodiri found that the wall of the conidium, like that of the hyphae, was composed of longitudinally oriented elements which gave rise to striations and was electronmicroscopically characterized by long, narrow electron-dense areas.

Crystalline inclusions have been observed in both plant and animal cells grown under varied conditions (Tandler, 1962; Thornton and Thiman, 1964; Cronshaw, 1964; Wills, 1965; and Shnitka, 1966). The inclusions

are part of various bodies (termed 'microbodies') consisting of a crystalline core bounded by a single membrane.

Shatkin and Tatum (loc. cit.) were first to report dense hexagonal crystalloids in the hyphae of N. crassa. However, they were unable to ascertain the presence of a limiting membrane.

Tsuda and Tatum (1961), in a chemical and cytological study of crystalline bodies in various strains of Neurospora, found them to consist of ergosterol. They advanced the hypothesis that the plates and bipyramids observed in their study were in fact alternative crystalline forms of ergosterol.

In addition to the hexagonal type of crystals reported by Tsuda and Tatum, (loc. cit.), Garjobst, et. al. (1965) observed by light microscopy, very long, needle-like crystals in slow growing strains of Neurospora (abn - 1 and abn - 2).

Tatum and Luck (1967), in a study concerning the ultrastructure of the needle-like crystals seen by Garjobst (loc. cit.), found them to consist of electron-dense rods packed in a parallel lattice. They appeared to be characteristic of the particular mutant strains (abn - 1, abn - 2).

Aggregations of vesicles in the matrix between the cell wall and plasma membrane (lomasomes) were reported by Girbardt (1958; 1961). Moore and McAlear (1961) coined the term "border bodies" to designate these structures. Further investigations revealed that they bore a resemblance to bacterial mesosomes. For this reason Zachariah and

Fitz-James (1967) proposed that lomasomes in fungi should be called mesosomes.

Originally, lomasomes were thought to be exclusive to the fungi; however, Manocha and Shaw (1964) observed lomasome-like structures in the mesophyll cells of "Khapli" wheat.

Although lomasomes have been found in a great spectrum of organisms (most of them fungi), little concrete evidence as to their exact function has been obtained. That these peripheral bodies play a major role in cell wall formation was suggested by Wilsenach and Kessel (1965). They noted that lomasomes appeared prior to the formation of cellular wall material in the matrix of the two membranes of the endoplasmic reticulum. The suggestion was made that the vesicles of the fungal lomasomes (like the Golgi bodies involved in cell plate formation) coalesce and form the cell wall. The writer acknowledges the above suggestion with some caution, since electron micrographs produced in the literature (including his own) do not allow for a definite statement with regard to the developmental sequealae involved.

Moore and McAlear (loc. cit.) observed granular and vesicular lomasomes in hyphae but failed to locate them in conidia and ascospores or in the basidiospores of the organisms studied. A mechanism for lomasome formation was suggested in which a vacuole moved from the cytoplasm to the cell periphery where it fused with the plasma membrane. A break in the fusion area would allow the expulsion of the contents. If the vesicular contents were aggregations of secreted ergastic or storage materials, the suggested secretory function of lomasomes may well be involved in the formation of the cell wall (Kozar and Weijer, 1969).

Shaw and Manocha (1965), investigating host-parasite relationships in wheat, suggested that encapsulement of the invading haustoria by the host may affect resistance of the parasite. He found that resistant wheat had a more rapid development of encapsulement. A secretory function of lomasomes was suggested since the resistant cells of "Khapli" wheat contained lomasomes whereas the parasite-susceptible varieties, such as "Little Club", were void of these structures.

Wells (1965), in a study concerning the ultrastructure of basidia and basidiospores of Schizophyllum commune, noted that vacuolating basidia contained aggregations of extracytoplasmic vesicles. The suggestion was made that these vesicles or lomasomes are involved in cytoplasmic degeneration.

On the basis of morphology and location of lomasomes, Zachariah and Fitz-James (loc. cit.) suggested as possible functions of the organelle a direct role in membrane synthesis, such as an involvement in the partitioning of the nucleoid and a mitochondrial role.

Fitz-James (1960) reported the existence of a lamellar or vesicular membranous structure in Bacillus medusa, B. megaterium and B. cereus. He coined the term "mesosome" to designate these structures. Recently Ryter (1968), reported similar complex lamellar and vesicular membranous structures in a number of a gram-positive bacteria. Since mesosomes originate as an invagination of the plasma membrane, Fitz-James (loc. cit.) proposed that their chemical and biochemical subunits are identical. Mesosomes in B. medusa consisted either of concentric membranes or of a complex vesicular structure.

Salton and Chapman (1962), observed in Micrococcus lysodeikticus clearly defined mesosome organelles, each consisting of a series of concentric membranes. Van Iterson (1961) showed the mesosome (either as a concentrically layered organelle or as a cluster of delimited vesicles) in contact with the cellular wall or situated in the proximity of the nuclear area.

Although little evidence is available for the existence of mesosomes in higher fungi, Zachariah and Fitz-James (loc. cit.) in Penicillium claviforme and Malhotra (1968) in the poky mutant of N. crassa reported mesosome-like structures. These structures were shown to be in direct continuity with the cytoplasmic membrane and resembling the mesosomes of bacteria.

Kozar and Weijer (1969) confirmed the existence of mesosomes in a St. Lawrence wild-type strain of N. crassa. These mesosomes resembled the mesosome of the fungus and are not restricted to the cytoplasmically aberrant mutant poky as suggested by Malhotra (loc. cit.).

The morphology of mitochondria may vary according to the organism, its stage of development and external influences. These variations in mitochondrial morphology can be expressed in the form, arrangement and number of cristae as well as the size and form of the organelles. Sussman (1966) showed that the morphology and population of the mitochondria varied during spore germination in Neurospora, while Butler and Bracker (1967) found that a similar variation occurred in the senescence portion of the life cycle of other fungi. Hawker (1965) and Luck (1963) have uncovered mitochondria in the filamentous fungi.

In addition to the above cellular organelles various other structures have been reported. Shatkin and Tatum (loc. cit.), described various cytoplasmic bodies, among them crystalloids and various particles, probably polysaccarides or lipids. Zalokar (loc. cit.), in a study concerning normal compared with centrifuged hyphae of Neurospora, added to the list of cellular constituents by describing glycogen, fat bodies vacuoles, ribosomes and various vesicles with inner canalicular systems.

Slautterback (1963) coined the term "microtubule" to designate a class of cellular structures, which had been referred to as filamentous elements, tubules or canaliculi in previous reports. These tubules ranged in diameter from 180 $\mathring{A}$ - 300 $\mathring{A}$ and are today considered a universal cell organelle. Although their exact function was not determined, Slautterback (loc. cit.), proposed on the basis of the confluence of these tubules with the vesicles of the Golgi complex, that they function in the transport of water ions or small molecules.

Behenke and Forer (1966) reported bridging material between intranuclear microtubules and chromosomes. They hypothesized that since bridging material is associated with the chromosome during cell division, these tubules play a role in chromosome movement (spindle).

Ledbetter and Porter (1963) reported cortical microtubules parallel to the cell surface and hypothesized that these exert an influence over the disposition of cell wall material besides playing a role in the governing of cytoplasmic streaming. Streaming is most rapid in the proximity of the cell cortex and the orientation of the long axis of the microtubules parallels the direction of streaming.

On the basis of the large number of cytoplasmic microtubules and evidence that these are in contact with other structures (Sandborn et. al., 1964) have suggested three specific functions: (1) supportive, (2) contractile (3) transport of fluids and suspended solids.

Other Organelles

(a) Cytolysome - like organelles.

DeDuve and co-workers (1959) are credited with originating the concept of the lysosome, a cellular organelle displaying acid phosphatase activity. Ashford and Porter (1962) observed that membrane-bound vacuoles contained various cellular organelles i.e., granules, mitochondria and fragments of ER all undergoing varying degrees of degeneration. Novikoff (1960) coined the term "cytolysome" to designate such vacuoles occurring in cells undergoing cytolysis. To date the presence of a similar organelle in N. crassa has not been unequivocally proven.

(b) Lipoprotein Sheets

Frey-Wyssling and Schwegler (1965) have described lipoproteinic sheets that store carotene. Rosso (1968) and Spurr and Harris (1968) have described "thylakoids" in their observations of β-carotene crystals as found in a number of varieties of tomatoes studied. Up to date, no lipoprotein sheets or thylakoids have been reported in Neurospora.

MATERIALS AND METHODS

Conidia of N. crassa, St. Lawrence Strain, mating type A were grown on minimal medium agar plates and incubated for 8-10 days at 25°C. Mature conidia were harvested by washing the agar surface of the plates with cold sterile distilled water and by subsequent filtration of the suspension through sterile glass wool in order to remove hyphal fragments. The harvested conidia were concentrated by centrifugation and

resuspended conidia were allowed to germinate in an incubator shaker to maintain aerobic conditions for varying periods of time (6 1/2 - 48 hours). The germinating conidia were collected on a Millipore filter and 1-mm squares with adhering germinating conidia were cut out of the conidial layer on the Millipore filter with a spear-head dissecting needle. These pellets were immediately fixed according to the schedule outlined below.

Fixation

Three methods of fixation were used in this study. The first method employed a double fixation of glutaraldehyde and osmium tetroxide. Glutaraldehyde was first used as a fixative for electron microscopy by Sabatini, Bensch and Barrnett, (1962). They found that glutaraldehyde was superior to several aldehydes tested. When compared to other common fixatives, glutaraldehyde enhanced the quality and amount of cytological detail observed. Sabatini et. al., (loc. cit.), found that double fixation--initial glutaraldehyde fixation, with post-fixation in osmium tetroxide--added contrast and further stabilized fine structures for electron microscopy.

Ledbetter and Porter (loc. cit.), using Sabatini's technique for double fixation, found that the plant cell cortex revealed minute tubuli and mitotic spindle structures hitherto unrevealed by conventional fixatives.

In this study glutaraldehyde was employed as a 2% solution in Sorenson's (0.15M) phosphate buffer pH 7.2 (24 hrs. at 20°C). The pellets were then rinsed in several changes of buffer to prevent the reduction of osmium by the glutaraldehyde. Prevention of osmium reduction

is necessary because reduced osmium is not effective as a fixative and, further, the reaction product coats the outside of the tissue extensively thereby reducing the rate of penetration of osmium tetroxide into the tissue. After rinsing, the pellets were post-fixed in a 2% solution of osmium tetroxide in phosphate buffer (0.15M) for 2 hrs. at room temperature.

Potassium permanganate was first used by Luft, (1956) as a fixative for electron microscopy. Later, Mollenhauer (1959) noted that good preservation of cell structure and all membrane systems was obtained using unbuffered solutions containing 2-5% $KMnO_4$ at room temperature. In the present study, pellets were transferred immediately after collection to the freshly prepared 2% solution of $KMnO_4$ and fixed for 2 hrs. at room temperature. A 2% solution of $KMnO_4$ for 30 mins. at room temperature was also employed in certain procedures.

The third method of fixation employed osmium tetroxide in 0.15M phosphate buffer. However, this technique was not used extensively since it did not provide as great a contrast nor maintain certain cell structures as well as double fixation.

Dehydration

Ethanol was used for dehydration throught this study. The material was dehydrated according to the following schedule:

30% ETOH	10 mins.
50% "	10 mins.
70% "	10 mins.
85% "	10 mins.

95 % ETOH 10 mins.

Abs. " 2 x 30 mins.

Embedding

Araldite was used as an embedding medium as suggested by Malhotra and Eakin (1967) with minor variations. After dehydration, the tissue was transferred to propylene oxide (two changes of 15 mins. eac). A 1:1 mixture of fresh propylene oxide and Araldite was poured over the material. The Araldite mixture consisted of:

DDSA (Dodecnyl succinic annhydride) 23 ml.

Araldite 502 27 ml.

DMP30 Dimethyl Amino Methyl Phenol (Accelerator) 1 ml.

The specimens were allowed to remain in covered vials (in a dust free area) for approximately 12 hrs. At the end of this period, the remaining Araldite-propylene oxide mixture was carefully drained off and a fresh Araldite mixture added. The specimens were allowed to remain in this mixture for 1 hr. They were then transferred into gelatine capsules filled with a fresh mixture of Araldite. The capsules were placed in an oven at 35° C for 1 hr. to allow air bubbles to rise to the surface. If necessary, tissue reorientation was also carried out at this stage. The capsules were then placed in an oven at 60° C for 48 hrs.

After polymerization and curing longitudinal, tangential and cross sections were cut from 600 to 1000 Å in thickness on a Porter-Blum microtome. Freshly cut glass knives were used and sections were collected on a 10 per cent solution of acetone in distilled water. These were expanded by chloroform vapors and picked up on formvar coated copper grids (200 mesh).

Staining

To increase the contrast of glutaraldehyde--osmium tetraoxide-fixed sections, grids were floated (section side downward) on droplets of stain placed on dental wax sheets. The specimens were stained in a 2% solution of uranyl acetate pH 4.8 (Huxley and Zubay 1961), for 5 1/2 hrs. This was followed by staining in lead citrate according to the method of Venable and Coggeshall (1965), for 5 minutes.

A similar procedure was used for staining the $KMnO_4$ - fixed sections. Sections were stained for 5 mins. in lead citrate according to the method of Venable and Coggeshall (loc. cit.), to enhance the contrast of the membrane system.

A Philips EM 100 B electron microscope with an objective aperature of 25μ was used for this study. Electron micrographs were taken on 35 mm Kodak film P426 at an initial magnifications of 4500-12,000X. These negatives were further enlarged photographically.

OBSERVATIONS

Potassium permanganate fixation

Transverse and longitudinal sections of both germinating and non-germinating conidia exhibited a coarse-grain cytoplasm bounded by a complex cell wall. The conidia were found to have a thick wall of relatively low electron density which, however, could be resolved into layers. Cytoplasmic and nuclear membranes and, in particular, those of the mitochondria were well defined. Although ribosomes were not preserved with this method of fixation, ER, consisting of elongated cisternae, vacuoles, (some containing electron-dense material), membranous bodies resembling lomasomes, complex concentric membranes (mesosomes), invaginations of the cell membranes as well as unidentified structures --- thylakoid-like sheets, lipid bodies and various other inclusions were well defined in many of the sections. $KMnO_4$ fixation brought out the membrane systems of the above organelles with exceptional clarity.

Glutaraldehyde and osmium tetroxide fixation

The ultrastructure of conidia fixed in glutaraldehyde and osmium tetroxide was well preserved. Walls of the conidia appeared thinner in this fixative than when fixed with $KMnO_4$. The well defined cell membrane made up of a unit membrane (although often irregularly invaginated along the surface of the conidium as in Figures 1, 7, 31, 35) was closely adherent to the cell wall. Elaborations of the invagination process may be seen in the formation of complex membrane systems consisting of concentric myelinoid type of rings (Figures 49, 56, 70). Double nuclear membranes containing pores were also well preserved, as were such organelles as mitochondria, vesicular bodies, ribosomes, lipid bodies, vacuolar membranes and microtubuli. In contrast to $KMnO_4$ fixation,

double fixation with glutaraldehyde and OsO_4 does not result in a well defined ER.

Although the fine structure of the germinating conidium (Figs. 1, 2, 3, 4, 5, 6, 7, 8) appeared to be similar to that of the ungerminated one, certain changes were manifested after the initiation of germination. The most prominent feature of the germinating conidium was the appearance of a large number of osmiophilic bodies usually associated with large vacuoles.

As germination continued the formation of septa was observed (Figs. 1, 5, 10, 48) which, when fully developed, consisted of a simple plate with a central pore unlike the complex pore type the so-called dolipore (Moore and McAlear, 1962). The germinating conidium also exhibited the typical cell organelles e.g., nucleus, mitochondria, ribosomes and ER as well as invaginations of the cell membrane (Fig. 1, 5, 7), microtubuli (Figs. 31, 32, 33, 34, 35, 36, 37), glycogen granules (Figs. 1, 8, 21, 30, 36), membranous structures (mesosomes) (Figs. 43-56, 57-60), border bodies or lomosomes (Figs. 38-42) and crystals (Figs. 21-24).

Endoplasmic Reticulum

Contrary to the findings of Zalokar (loc. cit.), this report confirms the findings of Manocha, (loc. cit.) who found that "soaked" conidia (as compared to "unsoaked" conidia) had well developed cisternae of ER. In the underlying study, evidence for a well developed ER was particularly pronounced in sections fixed in $KMnO_4$ (Figs. 11-20). The amount of ER present in a conidium varied from cell to cell. This variation may be attributed to the age and metabolic state of the cell. Great variation

was also exhibited in the patterns of branching of the ER (Figs. 10-12). Figures 11, 12, 14 show a lamellar ER consisting of two thin parallel membranes separated by a narrow space.

Crystalline Inclusions

Crystalline cytoplasmic inclusions, similar to those reported by Tatum and Luck (loc. cit.), were observed in wild type conidia of N. crassa. In longitudinal section (Figs. 21, 22, 23), they consisted of electron-dense rods (not unlike filaments arranged in a parallel fashion), while in cross section (Fig. 24) they exhibited a hexagonally packed pattern. None of the inclusions were bounded by a membrane. They were sparsely and randomly distributed in the cytoplasm.

Glycogen Granules

Glycogen, the primary polysaccaride in fungi (Zalokar, 1965), has a tendency to clump together in the form of aggregations (rosette or alpha particles) rather than being spread throughout the cytoplasm of the conidium or hyphae. In Figures 17, 18, 30, 66, 67 particulate clumped areas of varying intensity are shown in the cytoplasm. These particles are considered to be glycogen on the basis of fixation in $KMnO_4$ and subsequent staining in lead citrate.

Mitochondria

Mitochondria in both the conidia and hyphae showed variation in form and structure whether fixed in $KMnO_4$ or in glutaraldehyde followed by post-fixation in OsO_4. In many sections (Figs. 1, 4, 6, 22, 27) they appeared circular or elliptical in outline and hence these organelles are probably rod-shaped within the three dimensional cell. The arrangement of the cristae with respect to the longitudinal axis of the

mitochondria varied greatly and did not follow a particular pattern. It should be noted that in some organisms, (Euglena in particular) irregularly shaped mitochondria are common.

Vacuoles

Vacuoles of various sizes, delimited by a single unit membrane, were observed as electron-transparent areas in germinating conidia of N. crassa (Figs. 4, 7, 8, 14, 26, 37). This finding concurs with those of Weisz (loc. cit.), who reported large vacuoles in the conidia and hyphae of N. crassa which "often contained large dense bodies of unknown composition." Often irregular in outline, they frequently contained various stellate configurations of electron-dense material as well as membraned bodies and myelinoid-like configurations (Figs. 44, 53, 54, 55, 56). It has been suggested by Buvat and Carasso (1957), that vacuoles are formed by dilations of the ER. Thus the vacuolar membrane is regarded as a special-ization of the ER and in the present observation this is particularly apparent in $KMnO_4$-fixed sections.

The formation of vacuoles in the nuclear region by a similar mechanism can be seen in Figures 8,9, 13, 14, 15, 16. Serial sections 9a and 9b indicate a splitting of the double membrane of the nucleus. The ensuing dilation results in the formation of a vacuole.

In addition to the large vacuoles and their tonoplasts numerous smaller vesicles were also encountered (Figs. 4, 45, 45). These were frequently observed in relatively young cells.

Although microtubules were observed in juvenile as well as in mature cells, they were often seen circumventing the periphery of the large central vacuole so frequently found in young germlings. Figures 35, 36, 37 illustrate these microtubules.

Lomasomes

In this study cytoplasmic organelles associated with cell walls were observed in glutaraldehyde and OsO_4 - fixed germinated conidia of N. crassa (Figs. 38, 39, 41). These structures (lomasomes) were predominantly associated with vacuolating conidia and were always located at the periphery of the cell (conidia of N. crassa contained typical stellate vacuoles which colesced to form a large central vacuole). They appeared to consist of membrane-bound tubules and vesicles. At times they could be seen to be elaborations of the plasma membrane.

The present study has also shown lomasome-like structures (Figs. 40, 42) in conidia fixed in $KMnO_4$. These appeared to consist of membrane-bound tubules and vesicles and as in the sections fixed in glutaraldehyde and OsO_4, these were observed predominantly in association with vacuolating conidia. They were always observed at the periphery of the cell.

Mesosomes

Thin sections of germinating conidia of N. crassa, fixed in glutaraldehyde with post-fixation in OsO_4, have revealed the presence of ultracellular membranous structures similar to those reported by Fitz-James (loc. cit.). Both complex vesicular or canicular (Figs. 37, 43, 45, 51, 52) as well as concentrically-membraned organelles (Figs. 46, 47, 48, 49, 50, 56) were observed. These latter findings are in agreement with those reported by Fitz-James (loc. cit.), Glauert and Hopwood (1960) and Van Itersen (loc. cit.).

In Figures 13, 57, 58, 59, 60 mesosome-like structures are revealed in conidia fixed by $KMnO_4$. This is contrary to the findings of Curgy (1968) who, in a study concerning the ultrastructure of the hepatocytes of chick embryos, found myelin figures to be present only after double

fixation and never subsequent to $KMnO_4$ or OsO_4 fixation (exception cited - membranous whorls in cytoplasm).

The pronounced uptake of OsO_4 during fixation may indicate that these membranous structures (mesosomes) with their myelinoid-like configurations are high in lipid content.

Lipids and Spherosomes

As well as the glycogen granules, lipid droplets were found predominantly in germinating conidia as well as in mature or aged cells (Figs. 1, 5, 7, 52, 71). These elctron-dense bodies, whose chemical composition was not determined in this morphological study were considered to be fat bodies on the basis of their staining reaction. In germinating conidia particularly large osmiophilic bodies were often encountered (Figs. 5, 7). Since the number of small osmiophilic bodies in the germinating conidia was reduced, the large osmiophilic bodies may have arisen from the coalescence of the smaller lipid bodies.

A number of other particles resembling lipid droplets but showing a fine granulation were also observed. They, like the lipid droplets, were irregularly shrunken when stained with $KMnO_4$. Perner (1953) was among the first to name these structures "spherosomes" and pointed out the difficulty of distinguishing them from lipid inclusions (Perner, 1958). Thus the spherosome (on the basis of staining capacity) may be considered to be a special organelle endowed with the synthesis of lipids.

Electron Transparent Bodies

In addition to the above electron dense lipid droplets and spherosome-like organelles, a number of well defined electron transparent areas were observed in sections fixed in $KMnO_4$ (Figs. 13, 16) and in glutaraldehyde and osmium tetroxide (Fig. 29). A number of these were enclosed by a

single unit membrane. Clear areas were also observed in various sectors of the cell which were not membrane bound (Figs. 1, 21, 33). In certain cases, those electron transparent areas enclosed by a single membrane were observed to contain a few granular particles around their inner periphery.

Cytolysome-like organelles

On the basis of morphological analogy with cytolysomes in animal cells (Napolitano, 1963), the structures observed in Figures 68-74 were thought to be cytolysomes.

Lipoproteinic sheets

A number of what appeared to be lipoproteinic sheets were observed in germinating conidia. A serial section of one of these is shown in Figures 75, 76, 77.

DISCUSSION

The N. crassa conidium is a highly complex, often multi-nucleated fungal cell, possessing classical nuclear and cytoplasmic cellular components, characterized by profuse interconnecting membrane systems. The electron micrographs described in this report provide evidence to support Namboodiri's (loc. cit.), findings with respect to the morphology of the nucleus and nuclear membrane. Nuclei of the multinucleated conidia are bounded by an envelope comprised of the nuclear membrane proper, a perinuclear cisterna and an outer nuclear membrane. Evidence for the existence of a continuity between the outer nuclear membrane with the ER (first reported in animal cells by Watson (1955) and subsequently in plants by Shatkin and Tatum (loc. cit.), and Edwards (1960) can be seen in Figures 17, 18, 19, 20.

The outer nuclear membrane is continuous with the ER and numerous nuclear pores exist (whether or not these pores contain a thin membrane has not been unequivocally resolved to date) and should provide ample opportunity for the exchange of cellular materials between the nucleus and cytoplasm.

Shatkin and Tatum (loc. cit.), reported that nuclei can force their way through septal pores. Material fixed in $KMnO_4$ illustrates this process (Fig. 10). Serial sections indicated that the nucleus assumed a dumb-bell shaped form as it forced its way through the septal pore.

In an attempt to resolve the lack of uniformity in the literature with respect to the interpretation of the electron-light and dense areas of the nucleus, conidia fixed in 2% $KMnO_4$ for 30 mins. as well as for 2 hrs. were compared. Porter and Machado (1960) reported a direct correlation between the duration of fixation and the intensity of the

chromatic areas of the nucleus. Fixation in 2% $KMnO_4$ for up to 10 mins. resulted in chormosomes which were of lighter intensity than the remainder of the nucleoplasm. However, when the period of fixation was increased to 4 hrs. in 2% $KMnO_4$, a reversal of image intensity occurred. Hence, these results confirm those of Porter and Machado (loc. cit.). The micrographs described provide evidence that there is a correlation between the duration of fixation and the density of the electron image of the nucleus fixed in $KMnO_4$. The light areas in the nuclei of conidia fixed in 2% $KMnO_4$ for 30 mins., were interpreted as chromatic material. In contrast, an image reversal occurred in materials fixed for 2 hrs. in the same concentration of $KMnO_4$.

Endoplasmic Reticulum

Buvat and Carasso (loc. cit.) described large, clear areas bordered by a membrane analogous to that bordering a reticular formation and which dilated itself into a terminal bulb. Although the possibility of vesicular dilation of ER exists, Buvat and Carasso (loc. cit.), caution against the generalization that the vacuolar membrane might be regarded as a specialization of the ER. This report presents evidence which may support their hypothesis. Figures 13, 14, 15, 16 are sections illustrative of this process. Further evidence may be seen in Figures 42, 58.

The observation that ER was most prevalent in young actively growing hyphae may support the hypothesis that the form and abundance of the ER can be related to the developmental, physiological and environmental state of the conidia. The great variation exhibited in its patterns of branching would seem to indicate that ER is one of the most pleiomorphic organelles in N. crassa.

Morphological evidence indicates that ER is continuous with certain cellular organelles, e.g., nucleus and vacuoles (Figures 15, 19).

Crystalline Inclusions

It is difficult to determine from this study whether these membraneless inclusions were similar to the inclusions described in the microbodies of vertebrate liver (Shnitka, loc. cit.), or the crystalline containing bodies reported by Cronshaw (loc. cit.), or the intramitochondrial inclusions of normal liver parenchyma as described by Wills (loc. cit.).

Although Wills suggests that the presence of crystalline inclusions in a variety of diseases has been regarded as a non-specific degenerative phenomenon (they have also been found in normal tissue) no one theory can adequately explain their formation of function.

While the function of crystalline inclusions is uncertain much interest is being presently shown by investigators into their origin, structure, function and relationship to other organelles. King (1969), in his article on "Biocrystallography-An Interdisciplinary Challenge", cites Berval's (1965) belief "that the idea of life originating in an extended aqueous medium such as the sea, faces a serious and near-impossible barrier since such forms would be most diluted and least likely to survive in such a medium. Thus he has suggested that some physical process that will hold things together in an outer medium of fairly high dilution is indicated. This may be exterior by means of absorption on certain deposits, possibly of clay or iron oxides; or interior, on some spontaneously generated polymer." (p. 514)

King, contends that biocrystallography "holds great promise for the elucidation of the developmental aspects of organic form and structure". (p. 516)

Vacuoles

Weisz (1965) has hypothesized that the highly vacuolated conidia may represent an advanced stage in the life cycle of the conidium. Since osmiophilic bodies were always associated with large vacuoles and vesicular membrane systems, the complex thus formed may be the result of the conidium's mobilization of nutrients in the emerging germ tube. An alternate hypothesis may be put forth in which the loss of cytoplasmic constituents into the emerging germ tube may result in the formation of vacuoles.

The present observation of numerous small vacuoles in young fungal cells seem to support the hypothesis that large vacuoles are formed from the coalescence of the smaller ones.

Lomasomes

Contrary to the views of Zachariah and Fitz-James (1967), the author prefers to distinguish between lomasomes and mesosomes on the basis of their morphology and location. Mesosomes are myelinoid figures often concentrically arranged and frequently associated with large vesicles (Figs. 53-56) and mitochondria. Lomasomes, on the other hand, are membrane-bound tubules and vesicles located in the periphery of the cell. Since lomasomes have frequently been observed in contact with the cell wall, it is possible that these organelles perform a role in cell wall synthesis (Wilsenach and Kessel, 1965). In addition, observations in conidia of N. crassa point to a possible secretory function of lomasomes (Kozar and Weijer, 1969). Figure 38b suggests a vacuolar origin of the lomasome (a) and consequent fusion of the lomasome with the plasma membrane (b). Secretory products including the structural lomasome have been observed between the plasma membrane and the cell wall (c). These findings concur

with the views of Moore and McAlear (1961) with regard to a secretory function of the lomasome. The sequence of events leading to the expulsion of the lomasome and its contents can be diagrammed as in Fig. 81.

Mesosome

Although there are reports (Curgy, 1968) in the literature that mesosomes are possible artifacts which arise as the cell responds to the action of the fixative (glutaraldehyde and osmium tetroxide), others (Ryter, 1968) consider the mesosome to be a true entity with a mediating role between the nucleus and the membrane. In the study reported here, myelin-like structures such as mesosomes were identified in $KMnO_4$-fixed conidia as well and it appears unlikely therefore, that these structures are condensation configurations due to a particular fixative employed. According to Fitz-James (1966, 1967), the mesosomes contain precursors of the membrane and are, therefore, not stable organelles. Since to date, no mesosomes have been identified in the living cell, the existence of these organelles has not been unequivocally proven.

In N. crassa, both vesicular as well as lamellar types of mesosomes were observed and the question arises as to whether or not these two types of mesosomes represent different phases in the ontogeny of the organelle. The vesicular types of mesosomes appear to be somewhat similar to the structures described by Fitz-James (1960), Ryter and Jacob (1964) and Ryter (loc. cit.), in gram-positive bacteria consisting of a "pocket of cytoplasmic membrane in which tubules are rolled up, and the tubules are in turn invaginations of the membrane" (Ryter, 1968, p. 40). The lamellar type of mesosome in N. crassa resembles the one observed in gram-negative bacteria described by Ryter as structures not composed of

"rolled-up tubules but rather of folded or interdigitated membranes one upon the other." (p.42)

From the study to date, the function of the mesosomes in N. crassa is purely speculative. Whether or not they function in cell wall formation needs to be ascertained. The hypothesis might be advanced, however, that mesosomes are secretory organelles with a function somewhat similar to the Golgi apparatus, which, to date, has not been reported to be present in the higher fungi.

Lipids

The large osmiophilic bodies seen in the germinating conidia may have originated by the coalescence of smaller lipid bodies which appear to be frequently formed in germinating conidia. Fawcett (1966), suggests that these lipid bodies serve a two-fold purpose: (1) as a reservoir of high energy material and (2) a potential source of short chain hydrocarbons for the synthesis of membranes and other lipid bearing cell components.

Lipids synthesized within the cell appear to accumulate in the cytoplasmic matrix and not within membrane-limited aggregates. Those lipid bodies which appear to have a limiting membrane are characterized by a dense linear structure surrounding their periphery. This may be the result of a more intense reduction of OsO_4 at the interface between the cytoplasm and the lipid droplets.

The large osmiophilic bodies of varying configurations observed in vacuoles may have arisen at the "foci of physiological lysis" (Ashford and Porter, 1962). The formation of a membrane-enclosed vacuole is

followed by a subsequent lysis of the engulfed cellular components, some of which could well be lipid droplets (Figs. 69, 73).

Drawert and Mix (1962) were the first to distinguish spherosomes from lipid droplets, both of which reacted to the standard lipid dyes, varied in size and appeared irregularly shrunken as a result of fixation. Their finding disclosed that only the spherosomes when fixed with $KMnO_4$ exhibited a fine granulation. On the basis of this granulation (after fixation with $KMnO_4$) the organelles in Figures (61, 62, a and b, 63, 64, 65) are classified as spherosomes.

Frey-Wyssling and Mühlethaler (loc. cit.), suggests that if enzymatic activity is admitted the spherosome would not only be a simple precursor of oil droplets but a special organelle whose function is the production of fats.

Islam and Weijer (1969), in a genetic study dealing with the sexuality in N. crassa, reported that lipids are essential in the initiation and completion of the sexual cycle and constitutes the source of fatty acid (MW 400-600) which acts as a "sex inducing substance" in the process of cross-fertilization.

Electron Transparent Bodies

Apart from the cytoplasmic organelles so far described and discussed a number of relatively well defined electron transparent areas were observed in sections fixed in $KMnO_4$ (Figs. 30, 62) as well in glutaraldehyde and osmium tetroxide-fixed sections (Figs. 21, 33, 46, 74). Those enclosed by a single unit membrane may have been part of a vacuole. Those not so enclosed may represent areas of the N. crassa conidia which contained no unsaturated fatty acids. Therefore, the lack of reaction could result in

clear areas. In other areas (Figs. 33, 46, 62, 74) a few unsaturated fatty acids may have reacted to a limited degree, thus leaving characteristic clear spaces in the inclusion. Alternatively, the clear areas could be attributed to the lack of penetration of the fixative. Such a lack of penetration would result in the extraction of the lipid from the interior of the droplet during the subsequent stages of preparation, thereby giving rise to clear centers surrounded by a dense periphery of blackened lipid.

Microtubules

The function of microtubules as interpreted from the findings of this report is by nature, purely speculative. Ledbetter and Porter (loc. cit.) observed that when microtubules were in close proximity to the plasma membrane, a connecting substance was seen between the microtubule and the inner layer of the unit membrane. If this point of attachment anchored the microtubule, any undulation or other displacement forces along the length of the tubule would induce a streaming motion within the surrounding ground substance of the cytoplasm. The vigorous streaming of the protoplasm of N. crassa hyphae, as noted long ago by light microscopists, may have its origin in these undulations and displacement forces of the microtubule which are abundant in the fungal cell.

Cytolysome-like organelles

Napolitano (1963) suggests that in metabolically active adipose cells cytolysomes may originate in portions of the cell where catabolic enzyme localization has occurred. Ashford and Porter (1962) hypothesized that a membrane automatically forms around these areas of enzyme concentration thereby protecting the cell from autolysis. The writer

concurs with these views and suggests that the membrane forms following the accumulation of enzymes much in the same manner as the encapsulement of a pus pocket. Napolitano (loc. cit.), maintains that "the formation of cytolysomes might be regarded as a cellular process by which agents which threaten the integrity of internal cell structure are effectively isolated from the internal environment.

The formation of cytolysomes, then, may come about either as a prelude to cytolysis or as a result of an acute reorientation of normal metabolic process within the cell." (p. 480)

On the basis of morphological analogy to cytolysomes in animal cells, the structures shown in Figures 41, 68-74 are taken to be cytolysomes. Unequivocal proof would need to come from investigations employing acid phosphatase.

Lipoprotein Sheets

Frey-Wyssling and Schwegler (1965) reported that the over-production of carotene in the carrot root is stored in the form of lipoprotein sheets. They observed that when carotenes were produced during degeneration, they were not stored in "passive carriers resulting from a kind of lipophanerosis but in actively growing sheets" (p. 558). Since lipoprotein sheets were frequently observed in senescent conidia of N. crassa, it may be hypothesized that a similar process occurs in gerontic conidia of N. crassa.

The author acknowledges criticisms of his readers who may question his sweeping generalizations based solely on morphological analogies with other organisms. Encouragement for making these imaginative descriptions particularly, with respect to cytolysomes, falls due to the following

statement of Sjostrand (1965), with regard to the "art" of interpretation of electronmicrographs.

"The electron microscopist differs from most other research workers in one very obvious respect. When looking at his pictures, he becomes inspired to interpret the structural patterns in terms of function and inter-relationship of different structural components. His imagination, stimulated by these patterns, leads him to describe rather nice stories of functional sequences and events. In most other sciences, perhaps with the exception of certain branches of psychology, the idea resulting from this imaginary effort becomes the starting point of a series of experiments to prove or disprove it. Not so in electron microscopy. The attention such ideas have attracted seems to show that the mere fact that a picture can initiate an idea is considered proof that the idea is correct or that its inclusion in textbooks may be justified.

"The consequence is that different people get different ideas, and those who can put most artistic speculation and imagination into their interpretations will attract most attention ..... Therefore the electron miscroscopists' imaginative descriptions are very quickly picked up and passed on by physiologists, biochemists and so on. The pictures themselves have a sufficient quality of being obvious and concrete to give the impression of satisfactorily proving the validity of the speculations" (p. 47). It is the writers hope that biochemists, cytologists, and others will take up investigations to ascertain the validity of some of his speculations.

BIBLIOGRAPHY

Akai, S. and Ishida, N. 1968. An electron microscopic observation on the germination of conidia of Colletotrichum lagenarium, Mycopathol. et Mycol. appl. 34, 3-4: 337-345.

Ashford, T.P. and Porter, K.R. 1962. Cytoplasmic components in hepatic cell lysosomes. J. Cell. Biol. 12: 198-202.

Behnke, O. and Forer, A.F. 1966. Intranuclear Microtubules. Science 153: 1536-37.

Behnke, O. and Forer, A.F. 1967. Evidence for four classes of microtubules in individual cells. J. Cell. Sci. 2:169-192.

Bernal, J.D. 1965. Molecular matrices for living systems, p. 65-88. In the Origins of Prebiological Systems and of their Molecular Matrices, S. Fox, Editor. Academic Press, Inc., New York.

Butler, E.E. and Braker, C.E. 1965. Proc. Am. Phytopathol. Soc. Symp. Rhizoctonia solani.

Buvat, M.R. and Carasso, N. 1957. Mise en evidence de 1'ergstoplasme (réticulum endoplasmique) dans les cellules méri stématiques de la racine d'Allium cepa L: Compte rendu Acad. Sc. 244: 1532-1534.

Cairns-Smith, A.G. 1965. The origin of life and the nature of the primitive gene. J. Theoretical Biol., 10: 53-88.

Cronshaw, J. 1964. Crystal containing bodies of plant cells. Protoplasma. 59: 318-325.

Curgy, J.J. 1968. Influence du mode de fixative sur la possibilité d' observer des structures myéliniques dans les hépatocytes d' embryons de poulet. J. Microscopie. 7: 63-80.

De Duve, C. 1959. Lysosomes, a new group of cytoplasmic particles, in Subcellular Particles, C.T. Hayaslii, editor, New York, The Ronald Press Co. 128-159.

Drawert, H. and Mix, M. 1962. Die Sphärosomen in elektronen-mikrosopischen Bild. Ber. Deut. Botan. Ges. 75: 128-134.

Edwards, G.A. and Edwards, M.R. 1960. The intracellular membranes of Blastomyces dermatitidis. Am. J. Bot. 47: 622-632.

Edwards, M.R., Hazen, E.L. and Edwards, G.A. 1959. The fine structure of yeast-like cells of Histoplasma in culture. J. Gen. Microbiol. 20: 496-503.

Fawcett, D.W. 1966. An Atlas of Fine Structure. The Cell-Its Organelles and Inclusions. W.B. Saunders Co. Philadelphia.

Fitz-James, P.C. 1960. Participation of the cytoplasmic membrane in the growth and spore formation of bacilli. J. Biophys. Biochem. Cytol. 8: 507-528.

Fitz-James, P.C. 1966. The collection of mesosome vesicles extruded during protoplasting, p. 124-143. In L. Guze (ed), Symposium on microbial protoplast, spheroplasts and L-forms. Brook Lodge Conf. The Williams and Wilkins Co., Baltimore.

Fitz-James, P.C. 1967. A structural and functional comparison of separated fractions of mesosomes and plasma membrane of bacteria, Coll. Protides of biological fluids, Vol. 15. Elsevier Publishing Co., Amsterdam.

Frey-Wyssling, A. and Mühlethaler, K. 1965. Ultrastructural Plant Cytology with an Introduction to Molecular Biology. Elsevier Publishing Co., Amsterdam.

Frey-Wyssling, A. and Schwegler, F. 1965. Ultrastructure of the chromoplasts in the carrot root. J. Ultrastruct. Res. 13: 543-559.

Garnjobst, L., Wilson, J.F., and Tatum, E.L. 1965. Studies on a cytoplasmic character in Neurospora crassa, J. Cell Biol. 26: 413-425.

Girbardt, M. 1958. Uber die Substruktur von Polystictus versicolor L. Arch. Mikrobiol. 28: 255-259.

Girbardt, M. 1961. Licht-und electronmikroskopische Untersuchungen an Polystictus versicolor. II. Die Feinstruktur von Grundplasma und Mitochondrien, Arch. Mikrobiol. 39: 351-359.

Glauert, A.M., and Hopwood, D.A. 1960. The fine structure of Streptomyces coelicolor. I The cytoplasmic membrane system. J. Biophys. Biochem. Cytol. 7: 479-488.

Hawker, L.E. 1965. Fine structure of fungi as revealed by electron microscopy. Biol. Rev. 40: 52-92.

Huxley, H.E., and Zubay, G. 1961. Preferential staining of nucleic acid - containing structures for electron microscopy. J. Biophys. Biochem. Cytol. 11: 273-296.

Islam, M.S. and Weijer, J. 1969. In prep. A sex inducing substance in Neurospora crassa.

Kay, D.H. 1965. Techniques for Electron Microscopy, Blackwell Scientific Publications, Oxford.

King, L.J. 1969. Biocrystallography - An interdisciplinary challenge. Bio Science 19: 505-518.

Kozar, F. and Weijer, J. 1969. (a) Electron-dense structures in Neurospora I. Mesomes (in press).

Kozar, F. and Weijer, J. 1969. (b) Electron-dense structures in Neurospora II Lomosome-like structures (in press).

Ledbetter, M.C. and Porter, K.R. 1963. "A 'Microtubule' in plant cell fine structure". J. Cell. Biol. 19: 239-250.

Ledbetter, M.C. and Porter, K.R. 1964. Morphology of microtubules of plant cells. Science 144: 872-874.

Luck, D.J.L. 1963. Formation of mitochondria in Neurospora crassa, a quantitative radiographic study. J. Cell. Biol. 16: 483-499.

Luft, J.H. 1956. Permanganate - A new fixative for electron microscopy. J. Biophys. Biochem. Cytol. 2: 799-801.

Malhotra, S.K. 1968. Mesosome-like structures in Mitochondria of Poky Neurospora. Nature 219: 1267-1268.

Malhotra, S.K. and Eakin, R.T. 1967. A study of mitochondrial membranes in relation to elementary particles. J. Cell. Sci. 2:205-212.

Manocha, M.S. 1968. Electron microscopy of the conidial protoplasts of Neurospora crassa. Can. J. of Botany. 46: 1561-1564.

Manocha, M.S. and Shaw, M. 1964. Occurrence of lomasomes in mesophyll cells of "Khapli" wheat. Nature 203: 1402-1403.

Mollenhauer, H.H. 1959. Permanganate fixation of plant cells. J. Biophys. Biochem. Cytol. 6: 431-437.

Moore, R.T. and McAlear, J.H. 1961. Fine structure of mycota. V Lomasomes-previously uncharacterized hyphal structures. Mycologia 53: 194-200.

Moore, R.T. and McAlear, J.H. 1962. Fine structure of mycota VII. Observations on septa of ascomycetes and basidiomycetes. Am. J. Botany. 49: 86-94.

Namboodiri, A.N. 1966. Electron microscopic studies on the conidia and hyphae of Neurospora crassa. Caryologia 19: 117-133.

Napolitano, L. 1963. Cytolysomes in metabolically active cells. J. Cell. Biol. 18: 478-481.

Novikoff, A.B. 1960. Biochemical and staining reactions of cytoplasmic constituent, in Developing Cell Systems and Their Control, (D. Rudnick, editor), New York, The Ronald Press Co. 167-203.

Pease, D.C. 1964. Histological Techniques for Electron Microscopy 2nd ed. Academic Press, New York.

Perner, E.S. 1953. Die Sphärosomen (Mikrosomen) pflanzlicher Zellen. Profoplasma 42: 457-481.

Perner, E.S. 1958. Die Sphärosomen der pflanzenzelle. Protoplasmaforsch. 111/A/2 Springer, Wien.

Peveling, E. 1962. Der elektronenomikroskopische Nachweis der Sphärosomen in den Epidermiszelen der zwiebelschuppen von Allium cepa. Protoplasma 55: 429-435.

Porter, K.R. and Machado, R.D., 1960. Studies on the Endoplasmic Reticulum IV. Its form and distribution during mitosis in cells of onion root tip. J. Biophys. Biochem. Cytol., 7: 167-180.

Revel, J.P. Napolitano, L. and Fawcett, D.W. 1960. Indentification of glycogen in electron micrographs of thin tissue sections J. Biophys. Biochem. Cytol. 8: 575-589.

Rosso, S.W. 1968. The ultrastructure of chromoplast development in red tomatoes. J. Ultrastruct. Res. 25: 307-322.

Ryter, A. 1968. Association of the nucleus and membrane of bacteria: a morphological study. Bacteriological Reviews. 32: 39-54.

Ryter, A. and Jacob, J. 1965. Étude on microscope electronique de la liaison entre noyau et mésosome chez. B. subtilis. Ann. Inst. Pasteur 107: 384-400.

Sabatini, D.D., Bensch, K.G. and Barrnett, R.J. 1962. New fixatives for cytological and cytochemical studies. Proceedings Fifth Internatinnal Congress for Electron Microscopy, Philadelphia, 2: L3.

Salton, M.R.J. and Chapman, J.A. 1962. Isolation of the membrane-mesosome structures from Micrococcus lysodeikticus. J. Ultrastr. Res. 6: 489 - 498.

Sandborn, E., Koen, P.F., McNabb, J.D. and Moore, G. 1964. Cytoplasmic microtubules in mammalian cells. J. Ultrastruct. Res. 11: 123-138.

Shatkin, A.J. 1959. Morphology of Neurospora crassa. Trans. N.Y. Aca. Sc. II. 21(5): 446-453.

Shatkin, A. and Tatum, E.L. 1959. Electron microscopy of Neurospora crassa mycelia. J. Biophys. Biochem. Cytol. 6: 423-426.

Shaw, M. and Manocha, M.S. 1965. The physiology of host-parasite relations XV. Fine structure in rust-infected wheat leaves. Can. J. Bot. 43: 1285-1292.

Shnitka, T.K. 1966. Comparative Ultrastructure of hepatic microbodies in some mammals and birds in relation to species differences in uricase activity. J. Ultrastruct. Res. 16: 598-625.

Sjostrand, F.S. 1965. Critical evaluation of ultrastructural patterns with respect to fixation. In Symposia of the International Society for Cell Biology. Vol. 1. The Interpretation of Ultrastructure edited by R.J.C. Harris. Academic Press, New York.

Slautterbach, D.B. 1963. Cytoplasmic microtubules I. Hydra. J. Cell. Biol. 18: 367-387.

Spurr, A.R. and Harris, W.M. 1968. Ultrastructure of chloroplasts and chromoplasts in Capsicum annuum I. Thylakoid membrane changes during fruit ripening. Amer. J. Bot. 55: 1210-1224.

Sussman, A.S. 1966. Dormancy and spore germination. In The Fungi. Edited by G.C. Ainsworth and A.S. Sussman, Vol. 2. Academic Press. pp. 733-764.

Tanaka, K. and Yanagita, T. 1963. Electron microscopy on ultrathin sections of Aspergillus niger. II. Fine structure of conidia-bearing apparatus. J. Gen. Appl. Microbiol. 9: 189-203.

Tandler, C.J. 1962. Bound Indoles in Acetabularia. Planta. 59: 91-107.

Tatum, E.L. and Luck, D.L.J. 1967. Nuclear and Cytoplasmic Control of Morphology in Neurospora. Developmental Biology Supplement 1, 32-42.

Thornton, R.M. and Thimann, K.V. 1964. On crystal-containing body in cells of the oat coleoptile. J. Cell. Biol. 20: 345-50.

Toner, P.G. and Carr, K.E., 1968. Cell Structure, An Introduction to Biological Electron Microscopy. E. and S. Livingston Ltd. Edinburgh.

Tsuda, S. and Tatum, E.L. 1961. Intracellular crystalline ergosterol in Neurospora. J. Biophys. Biochem. Cytol. 11: 171-177.

Van Iterson, W. 1961. Some features of a remarkable organelle in Bacillus substilis. J. Biophys. Biochem. Cytol. 9: 183-192.

Venable, J.H., and Coggeshall, R. 1965. A simplified lead citrate stain for use in electron microscopy. J. Cell.Biol. 25: 407-408.

Vogel, H.J. 1956. A comment on growth medium for Neurospora crassa. Microbiol. Genet. Bull., No. 13, 42-43.

Walek-Czernecka, A. 1962. Mise en évidence de la phosphatase acide (monophosphoesterase 11) dans les sphérosomes des cellules épidermiques des écailles bulbaires d'Allium cepa. Acta Soc. Bot. Plan. 31: 539-543.

Watson, M.L. 1955. The nuclear envelope. Its structure and relation to cytoplasmic membranes. J. Biophys. Biochem. Cytology. 1: 257-70.

Weisz, B. 1965. An electron microscope and biochemical study of Neurospora crassa during development. J. Gen. Microbiol. 39: 85-94.

Wells, K. 1965. Ultrastructural features of developing and mature basidia and basidiospores of Schizophyllum commune Mycologia 57: 236-261.

Wills, E.J. 1965. Crystalline structures in the mitochondria of normal human liver parenchymal cells. J. Cell. Biol. 24: 511-514.

Wilsenach, R. and Kessel, M. 1965. The role of lomasomes in wall formation in Penicillium claviforme. Can. J. Microbiol. 40: 401-404.

Zachariah, K., and Fitz-James, P.C. 1967. The structure of phialides in Penicillium claviforme. Can. J. Microbiol. 13: 249-256.

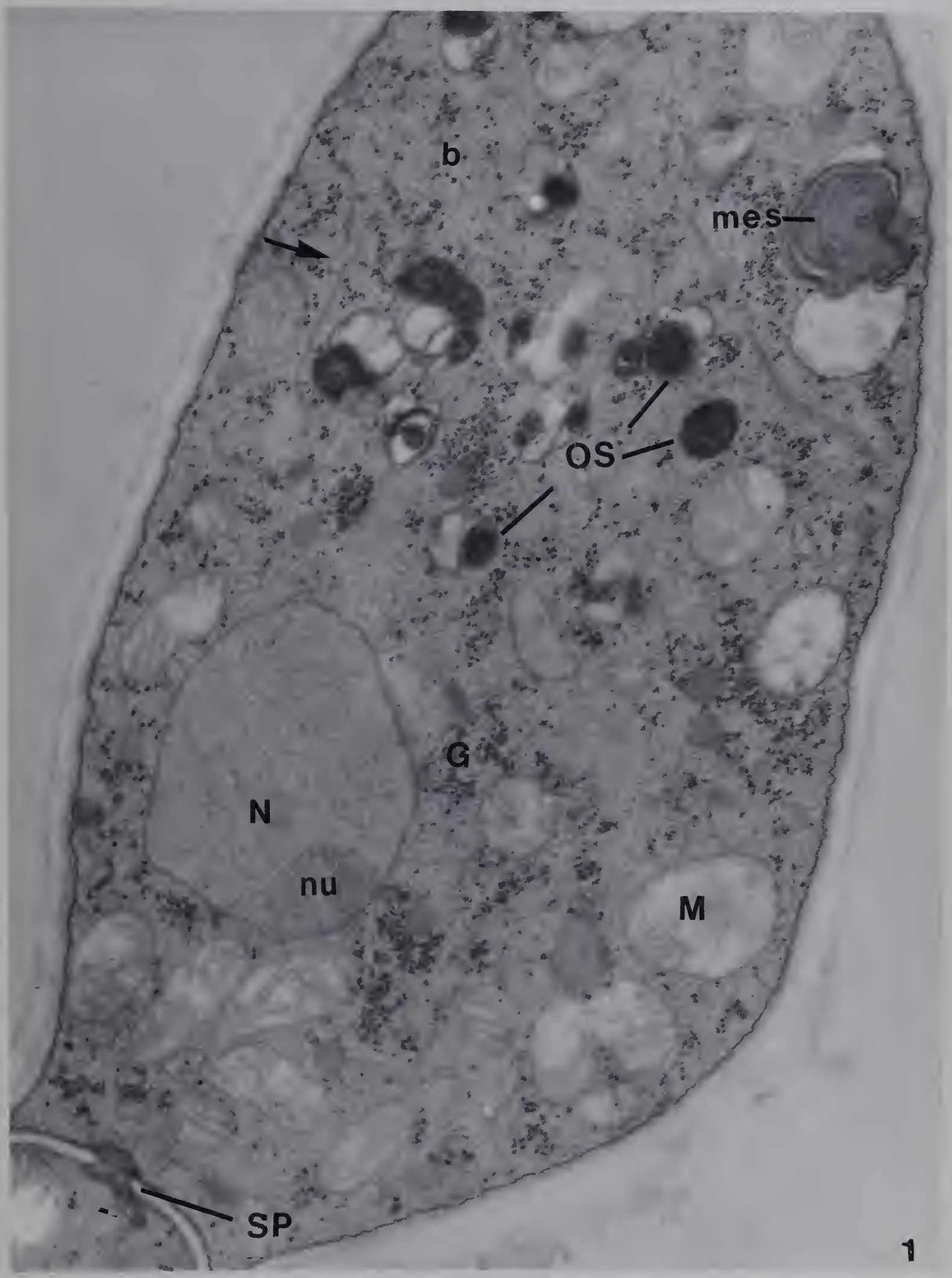
Zalokar, M. 1961. Electron microscopy of centrifuged hyphae of Neurospora. J. Biophys. Biochem. Cytol. 9: 609-617.

Zalokar, M. 1965. Integration of cellular metabolism. In The Fungi Edited by G.C. Ainsworth and A.S. Sussman. Vol. 1 Academic Press, New York. pp. 377-426.

KEY TO FIGURES

b	beta particles	V	vacuole
ch	chromosomes	ves	vesicle
Cr	crystalline inclusions	w	wall
cyt	cytolysome		
DM	double membrane		
ER	endoplasmic reticulum		
ET	electron transparent area		
G	glycogen - alpha particles or rosettes		
GT	germ tube		
lo	lomasomes		
M	mitochondria		
mes	mesosomes		
MT	microtubule		
N	nucleus		
nb	nuclear "bleb"		
np	nuclear pore		
nu	nucleolus		
OS	osmiophilic		
pm	plasma membrane		
R	ribosomes		
Se	septum		
SM	single membrane		
SP	septal pore		
sph	spherosome		
V	vacuole		

Figure 1. The micrograph illustrates a germinating conidium of N. crassa fixed in glutaraldehyde and osmium tetroxide and double-stained in uranyl acetate and lead citrate.
Glycogen can be seen as numerous beta particles (b) occurring singly in the cell or in the form of aggregates of larger size, alpha particles or rosettes (G).
Arrow indicates a microtubule.
Also seen are various osmiophilic bodies (OS) some of which are parts of vacuoles. These may coalesce to form the large osmiophilic bodies frequently seen in the germinating conidium. In addition the micrograph shows a nucleus (N), nucleolus (nu), mesosome (MES), a septal pore (SP) and mitochondria (M).
X 43,000



1

Figure 2. $KMnO_4$-fixed germinating conidium illustrating the multinucleated condition (N). Numerous small vesicles (ves), some of which may be terminal dilations of the endoplasmic reticulum (ER), are also shown both in the germ tube (GT) and in the conidium. These may coalesce to form a large vacuole. The light areas in the nucleus are interpreted as chromosomes (ch). Lead citrate-stained.

X 35,500

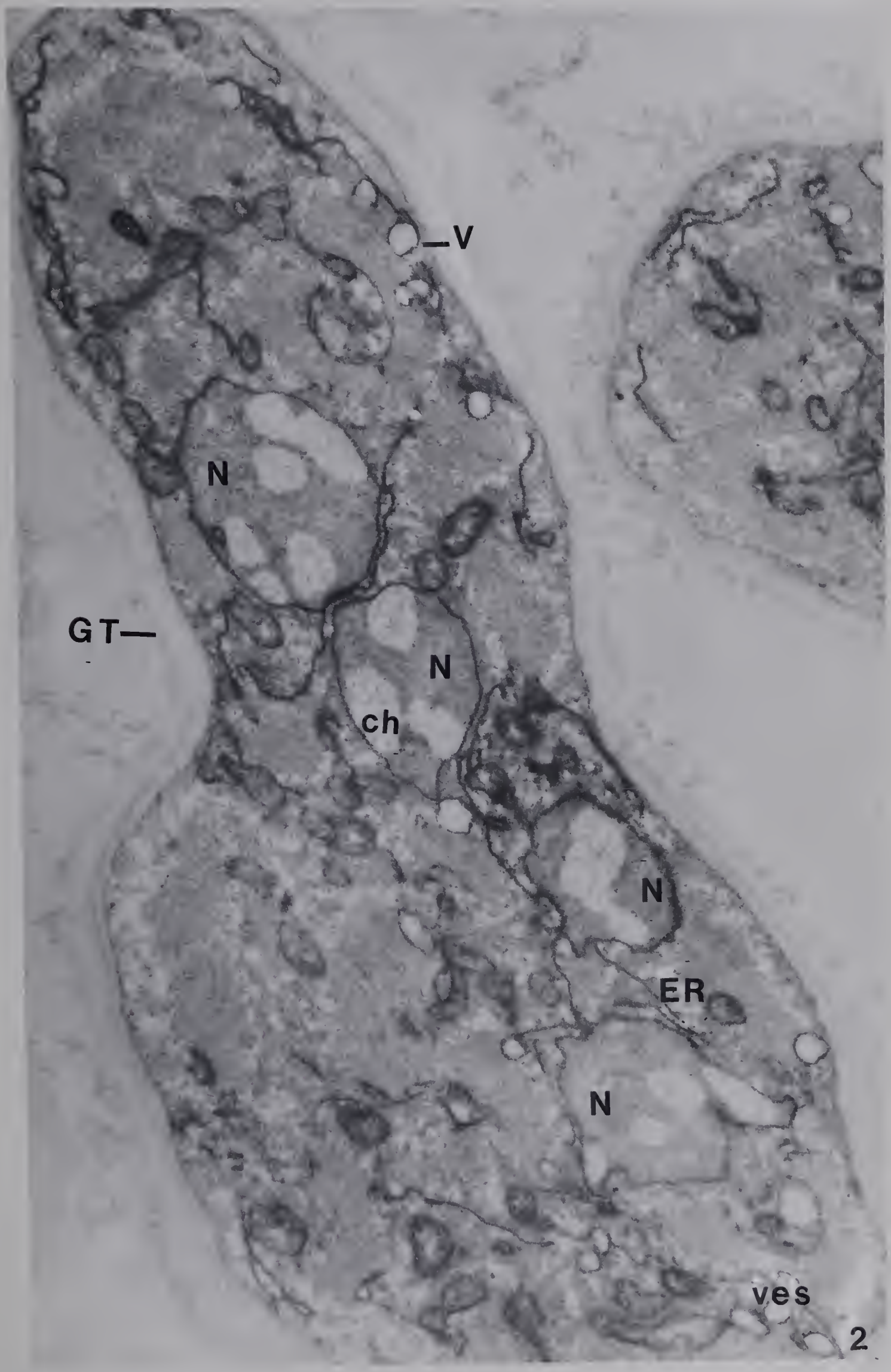
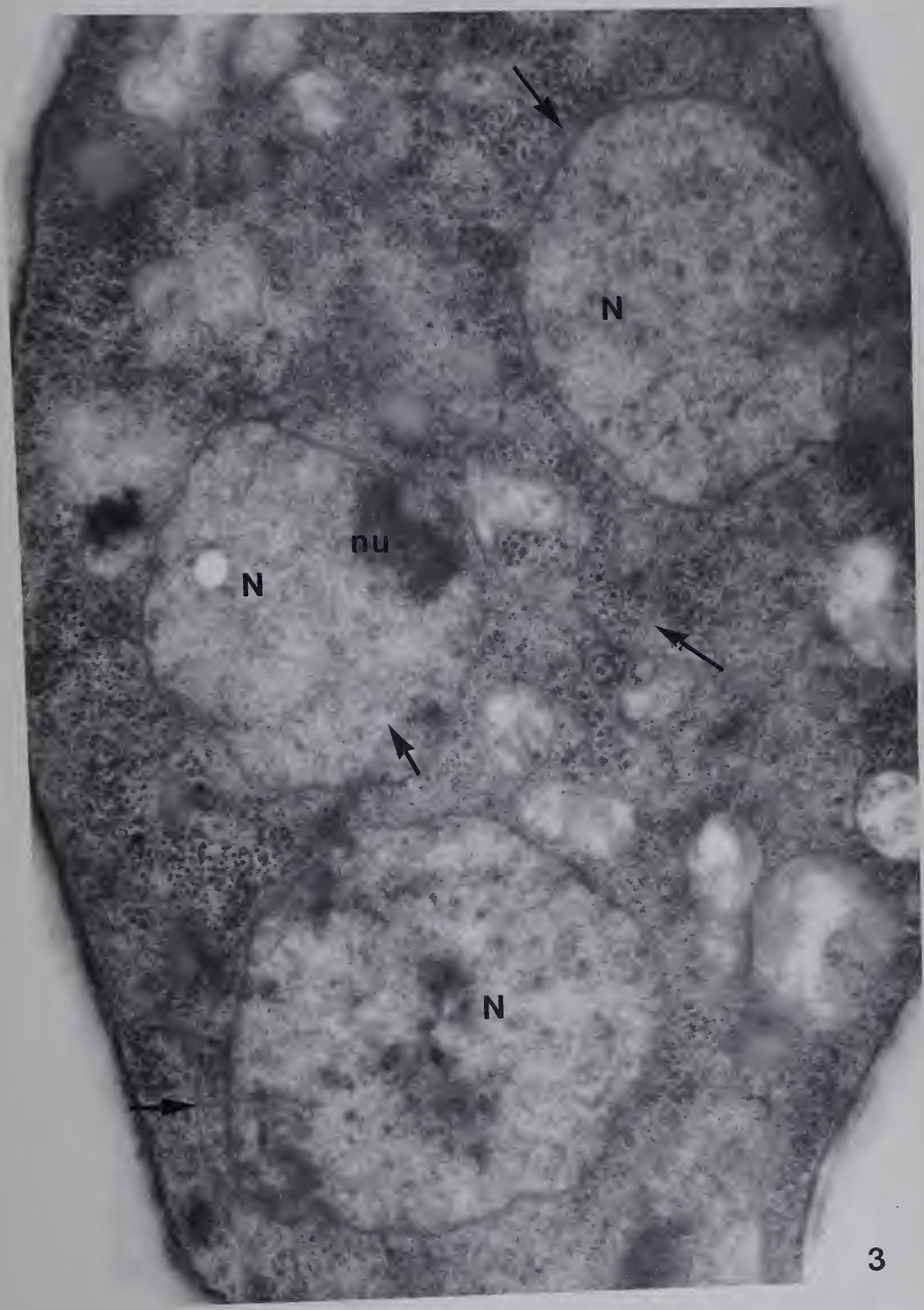

V
N
GT
N
ch
N
ER
N
ves
2

Figure 3. An enlarged portion of a germinating conidium of N. crassa illustrating the multinucleated condition frequently observed. Also shown are numerous microtubules (arrows). These microtubules may be a resilient cytoskeleton, playing a role in the maintaining of cellular shape by imparting rigidity to certain areas of the cell.

Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate and lead citrate staining.

X 80,000



3

Figure 4. The micrograph illustrates the formation of a large vacuole (V) by the coalescence of a number of smaller vesicles (ves). A number of microtubules (MT) are seen in lower right hand section of the micrograph. Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate and lead citrate-stained.

X 63,000

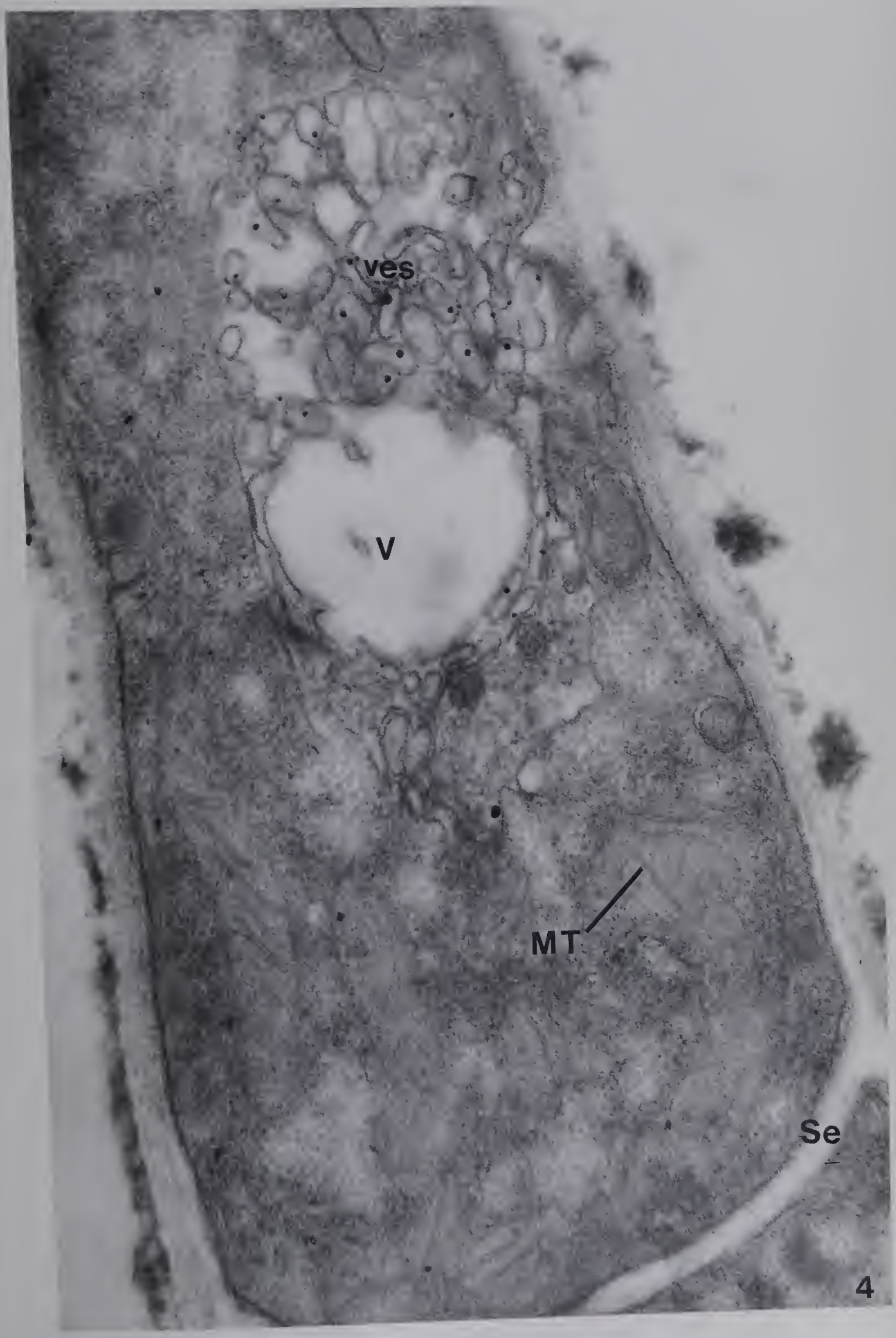

ves
V
MT
Se
4

Figure 5. Longitudinal section of a germinating conidium of N. crassa illustrating cytoplasmic details. Complex concentric membranes, mesosomes (mes), are frequently found near the septal pore (SP). Also seen are a number of osmiophilic bodies enclosed in vacuoles. These may coalesce to form large osmiophilic bodies.

Glutaraldehyde and osmium tetroxide fixation. Stained with uranyl acetate and lead citrate.

X 54,800

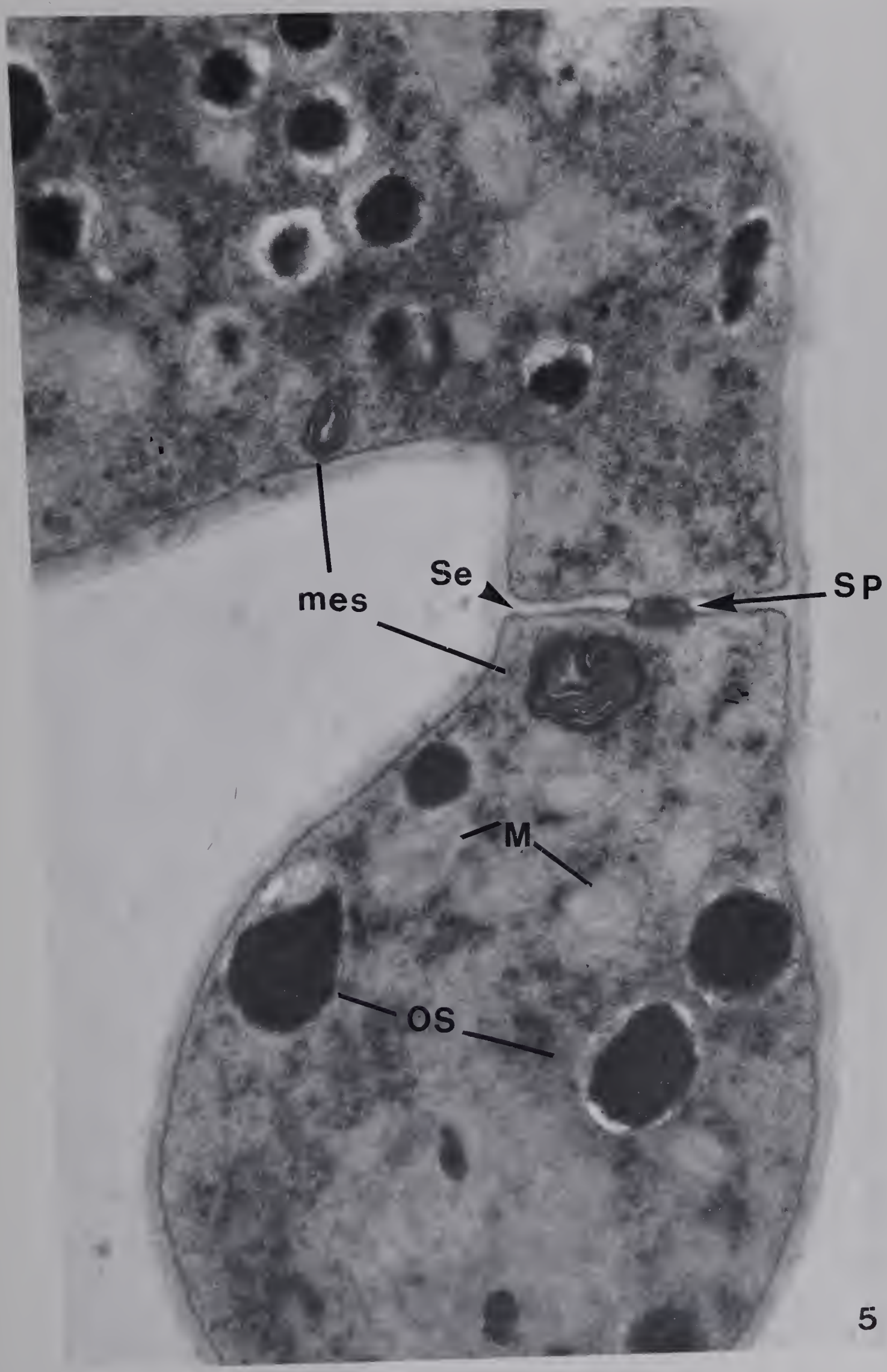
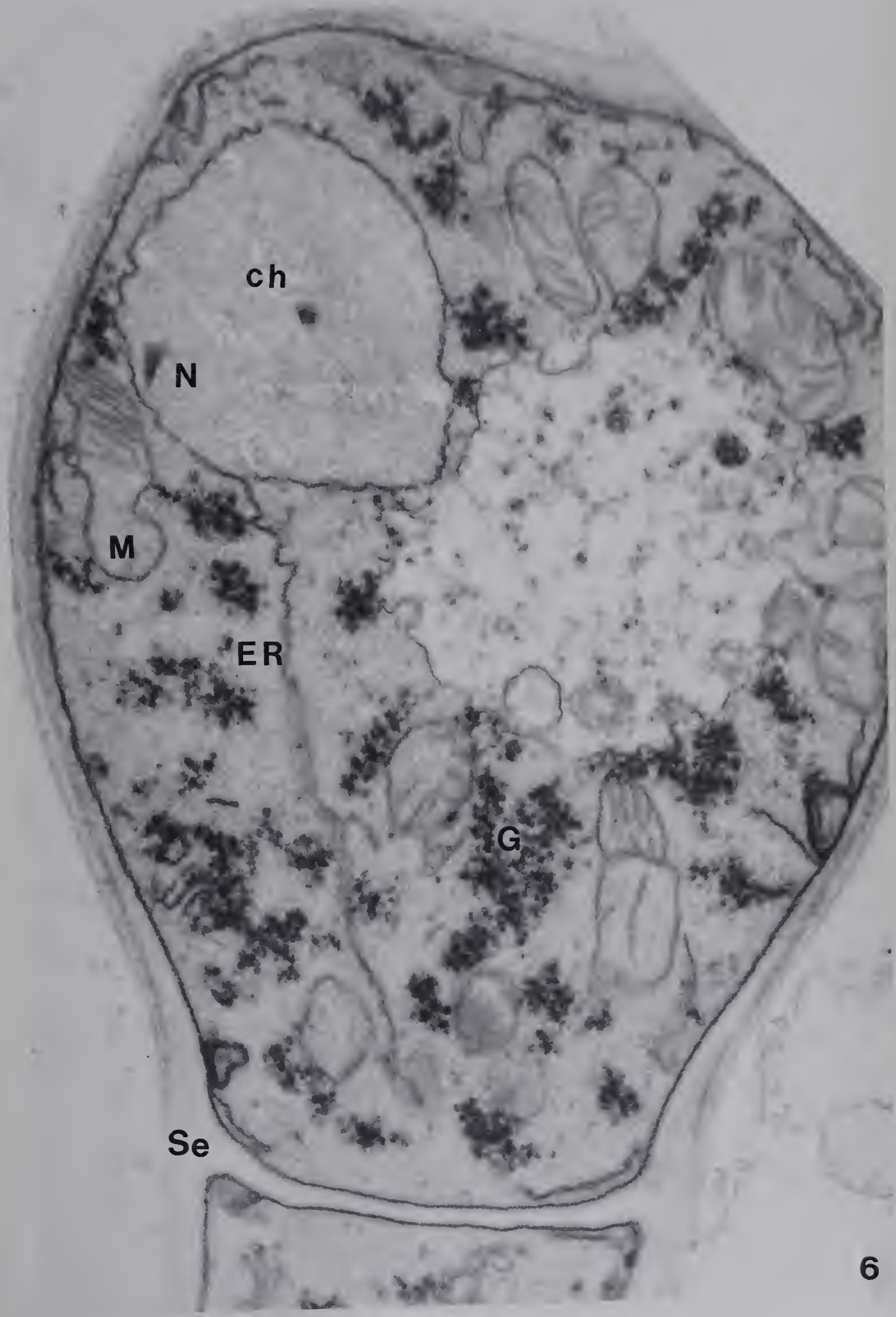

mes
Se
SP
M
OS

Figure 6. Longitudinal section of a germinated conidium of N. crassa illustrating the continuity of the nuclear envelope with the ER. The electron-light areas in the nucleus are interpreted as regions of chromatic material. A stellate vacuole and numerous rosettes of glycogen (G) can also be seen.

$KMnO_4$ - fixed; lead citrate-stained.

X 48,000



6

Figure 7. Longitudinal section of a germinating conidium of N. crassa illustrating cyloplasmic details. The germ tube contains the tubular form of a mesosome (mes). Other notable features are the large osmiophilic bodies, with their associated vacuoles (v) and mitochondria (M). Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate and lead citrate-stained. X 72,000

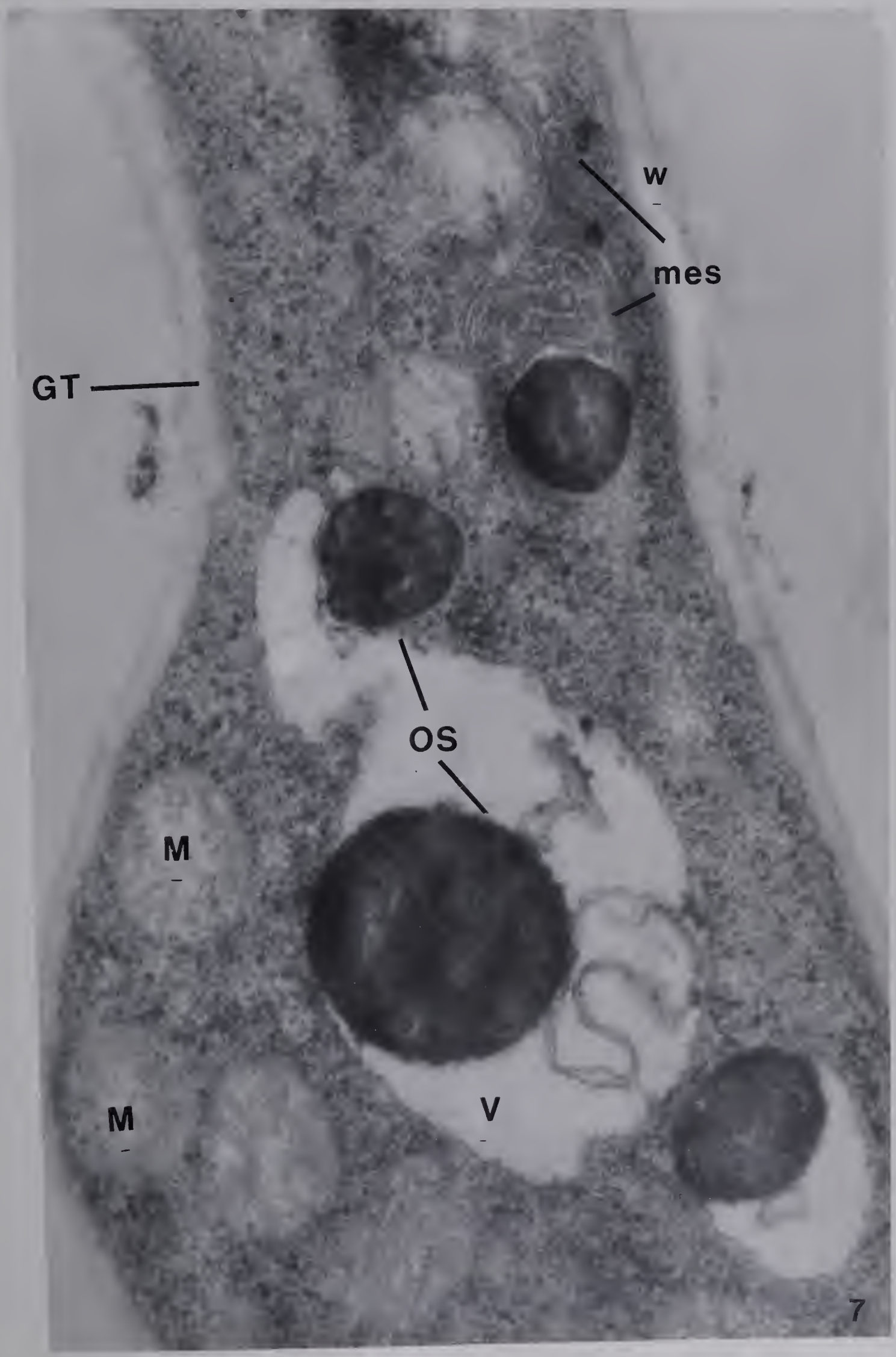
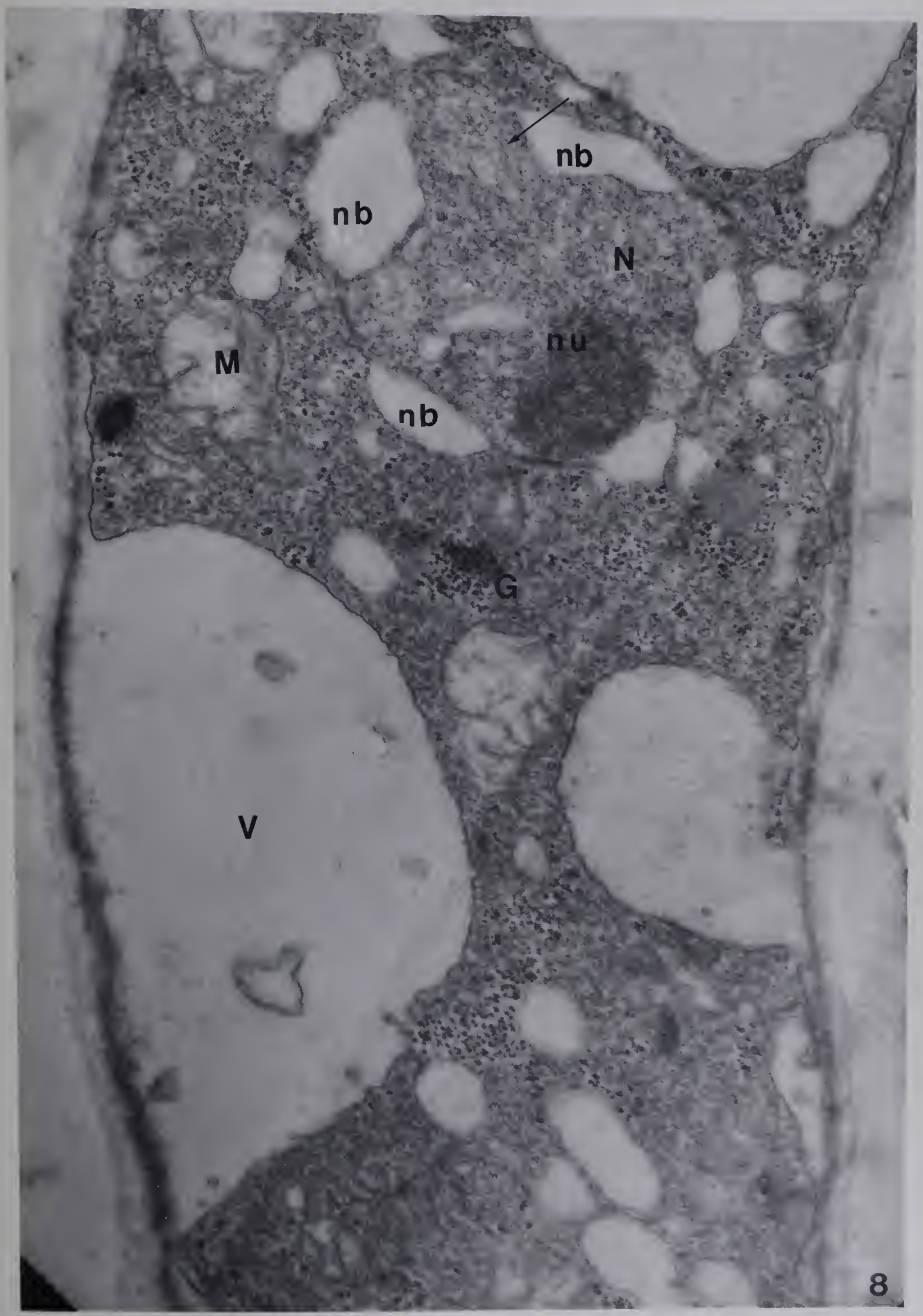

w
mes
GT
OS
M
M
V
7

Figure 8. Maturing hyphal longitudinal section of N. crassa illustrating the highly vacuolation condition of maturing hyphae. The nucleus contains numerous "nuclear blebs" (nb) formed by the separation of the nuclear membrane. The nucleolus (nu) is stained darkly by the action of uranyl acetate and lead citrate. A number of microfilaments (arrow) can be seen in the nucleus (N).

Glutaraldehyde and osmium tetroxide fixation.

X 42,000



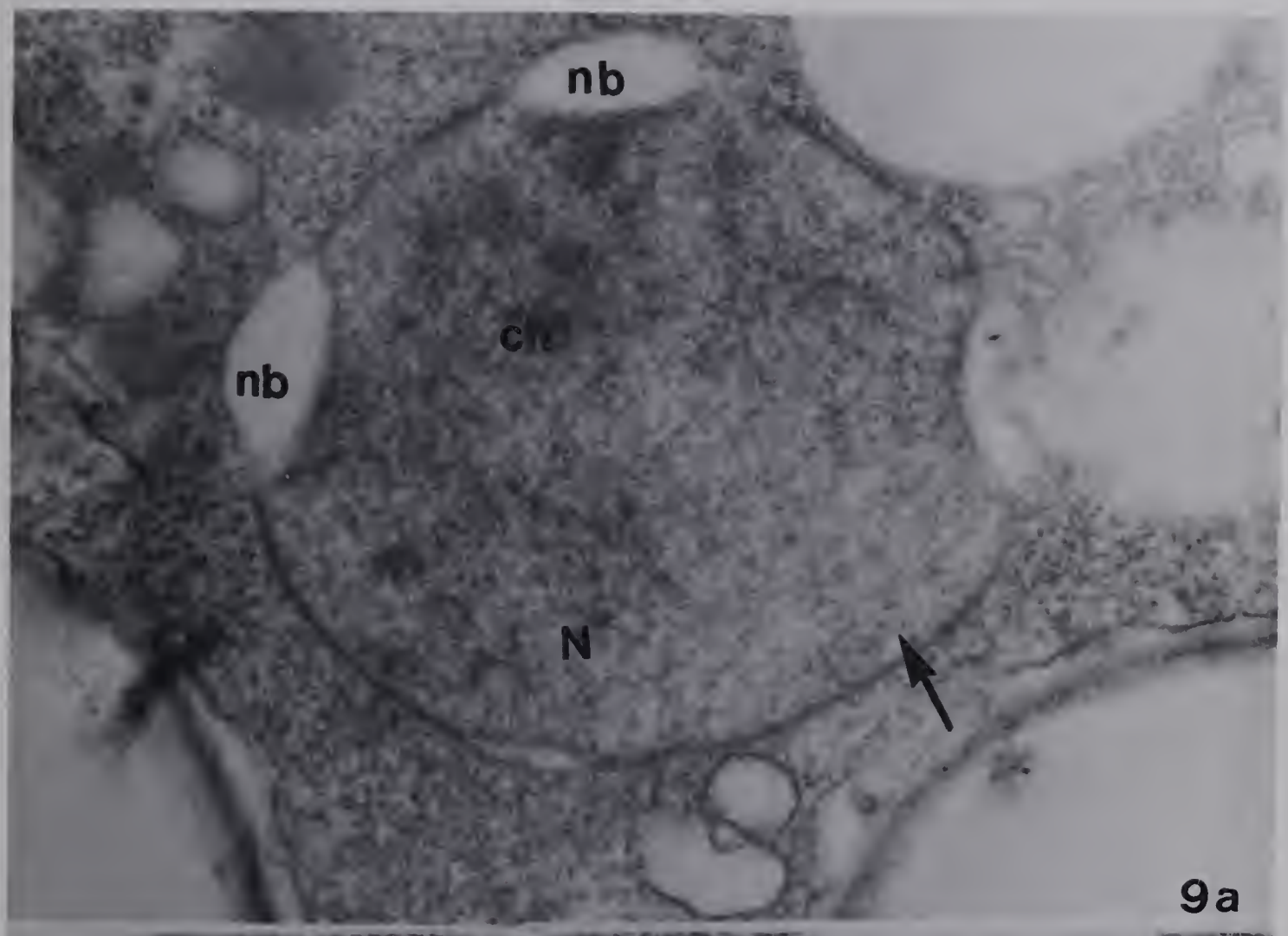
8

Figures 9a and 9b. Serial sections of a germinating conidium illustrating "nuclear blebs" (nb). These configurations are formed by a separation of the nuclear envelope. Arrows indicate microtubules in the nucleus (N).

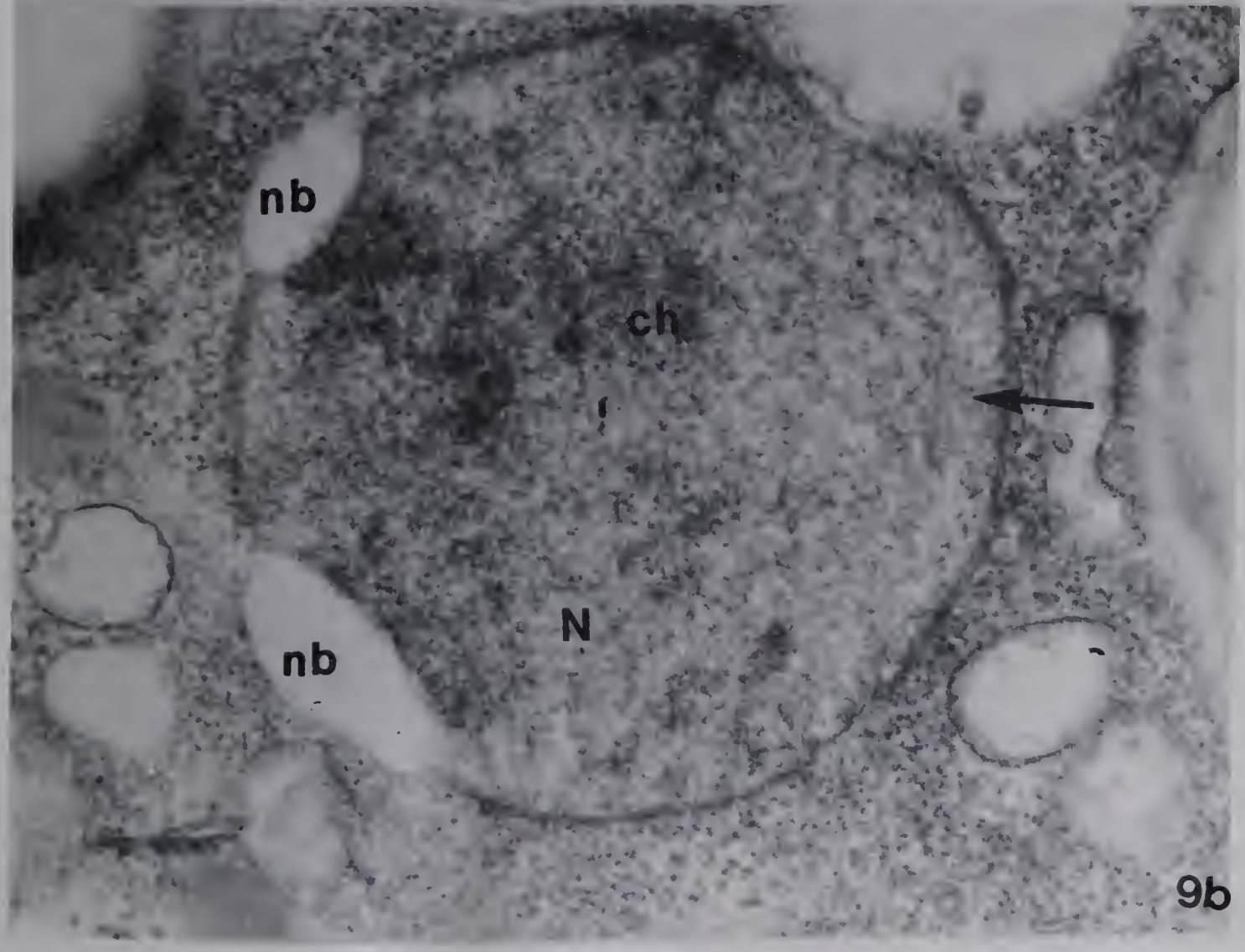
Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate-stained.

Figure 9a X 52,000

Figure 9b X 61,500



9a

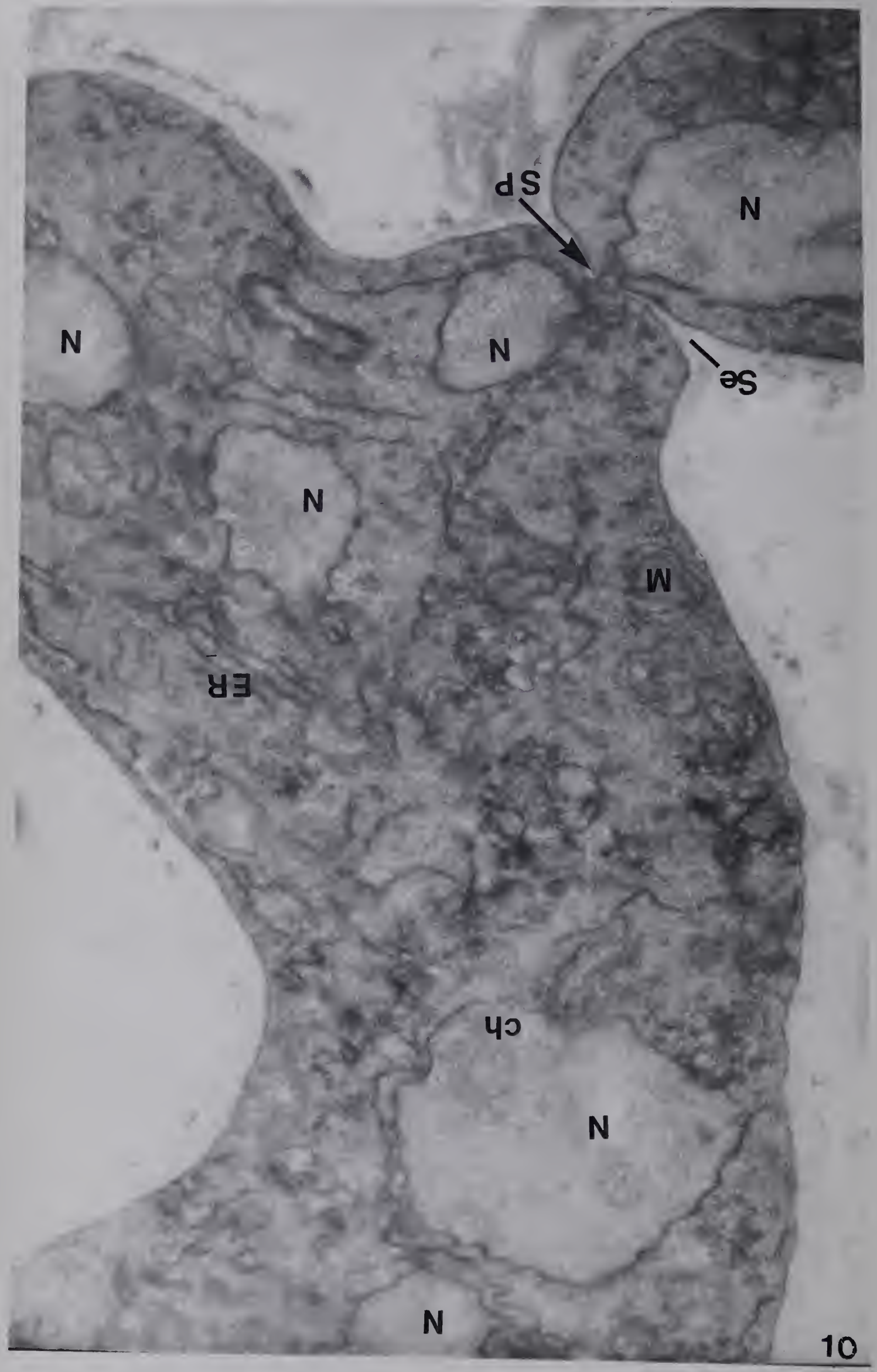


9b

Figure 10. Longitudinal section of a hyphae showing the multinucleated condition of N. crassa. Several sections revealed that the nucleus has the capacity to pass through the complex sepal pore (lower left). The pleiomorphic nature of the ER can be seen in the great variation exhibited in its patterns of branching.

$KMnO_4$-fixed; lead citrate-stained.

X 58,000

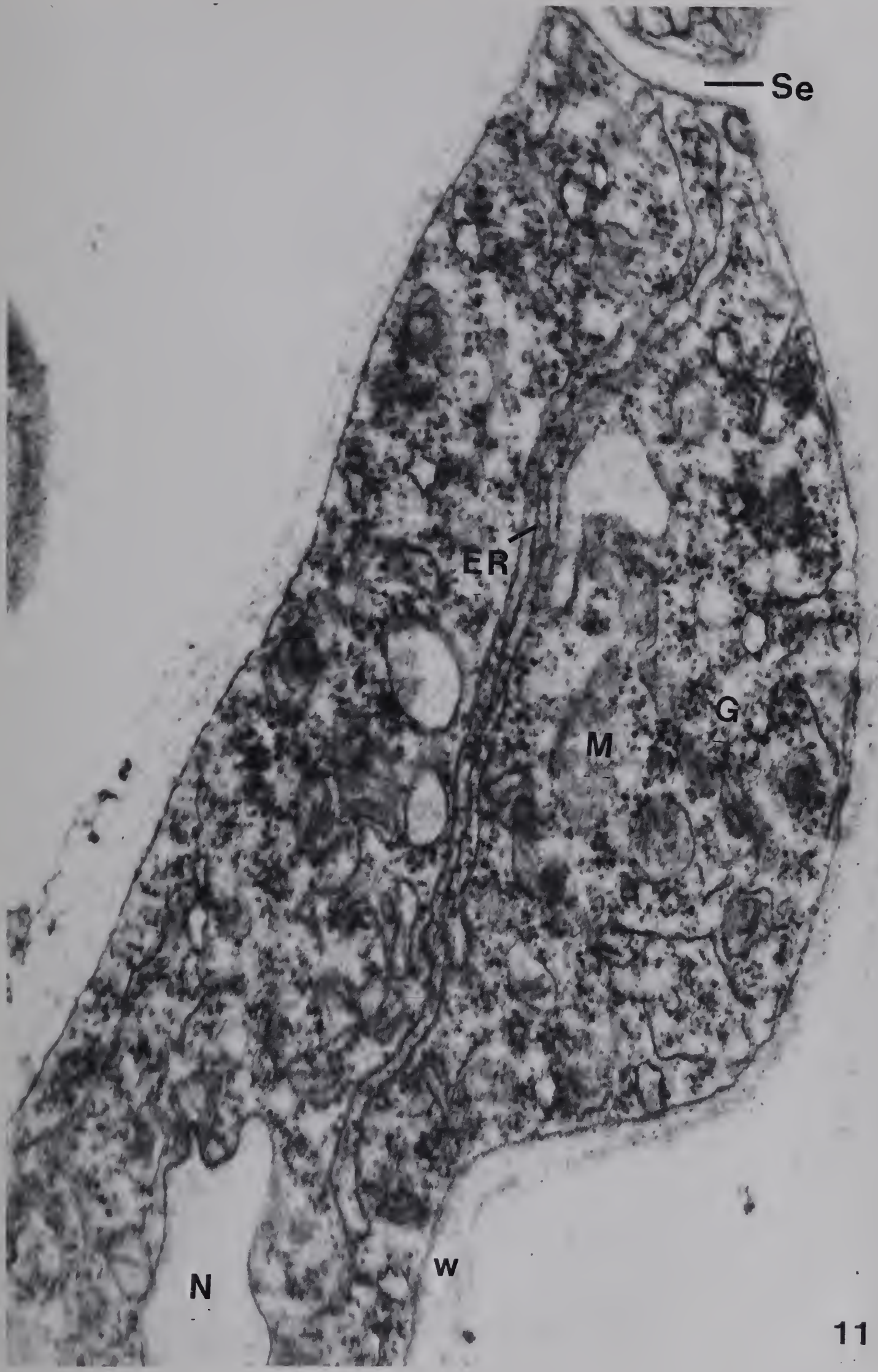


10

Figure 11. Longitudinal section of a germinated conidium showing a lamellar type of endoplasmic reticulum (ER) traversing the length of the section seen. Numerous aggregations of glycogen (rosette particles) (G) can also be seen.

$KMnO_4$ - fixed, lead citrate-stained.

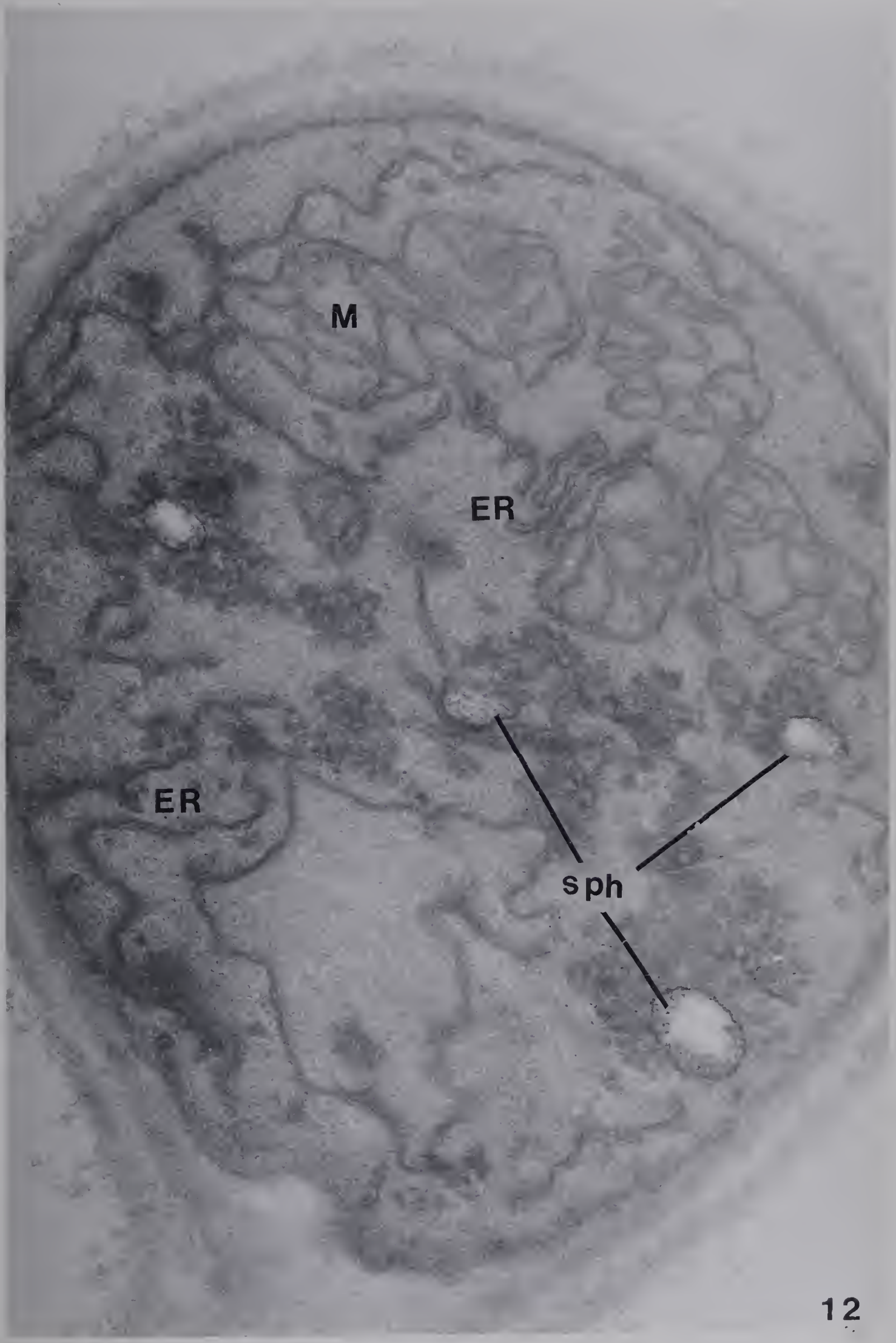
X 54,000



11

Figure 12. A conidium fixed in $KMnO_4$ and stained with lead citrate showing oldly shaped mitochondria (M) and endoplasmic reticulum (ER) in various configurations. Also shown are several spherosomes (sph).

x 72,300

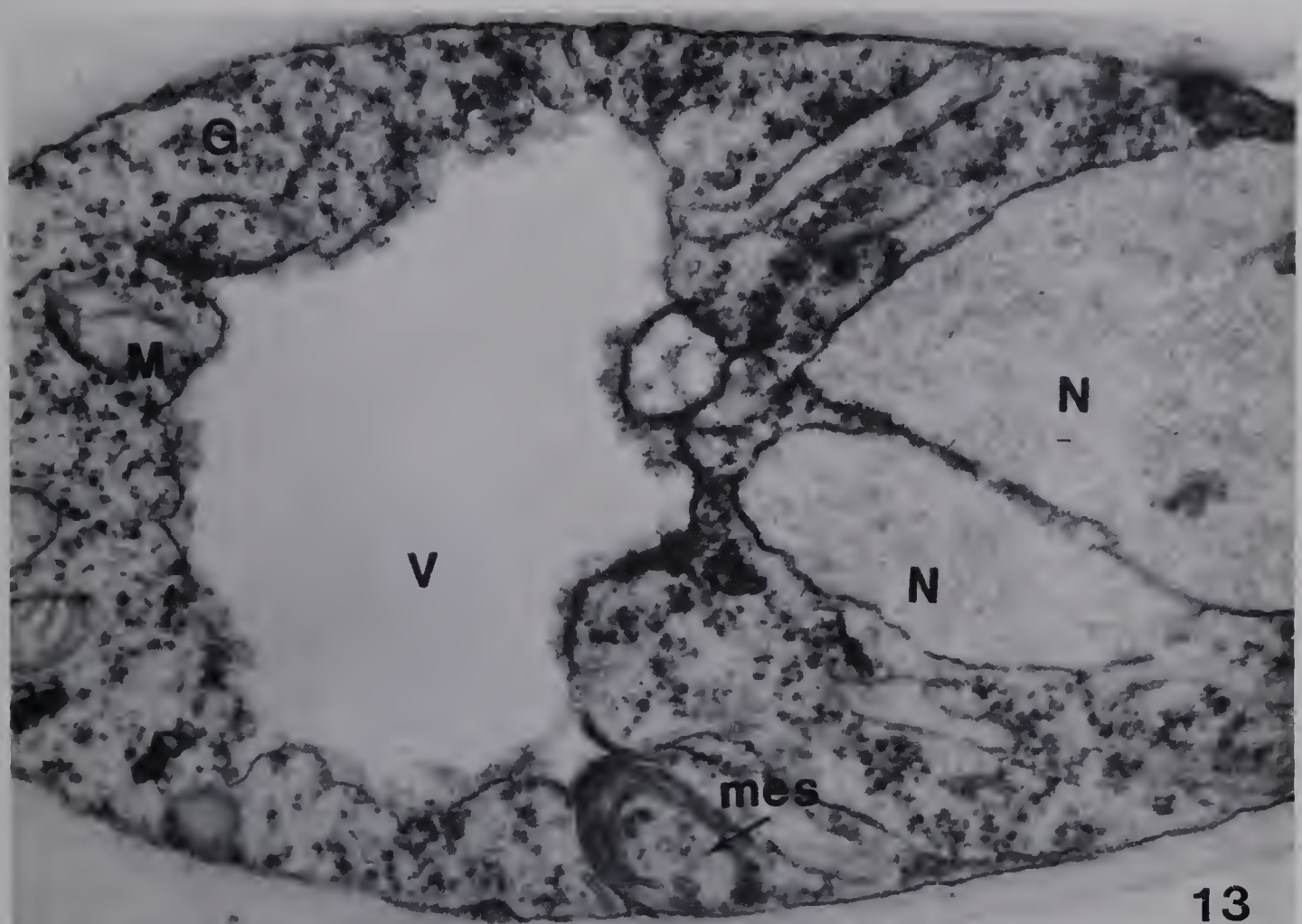

M
ER
ER
sph

Figures 13 and 14. Dilation of the endoplasmic reticulum in the formation of vacuoles (V). In addition, Figure 13 shows a mesosome (mes) near the periphery of the conidium while Figure 14 shows several spherosomes (sph).

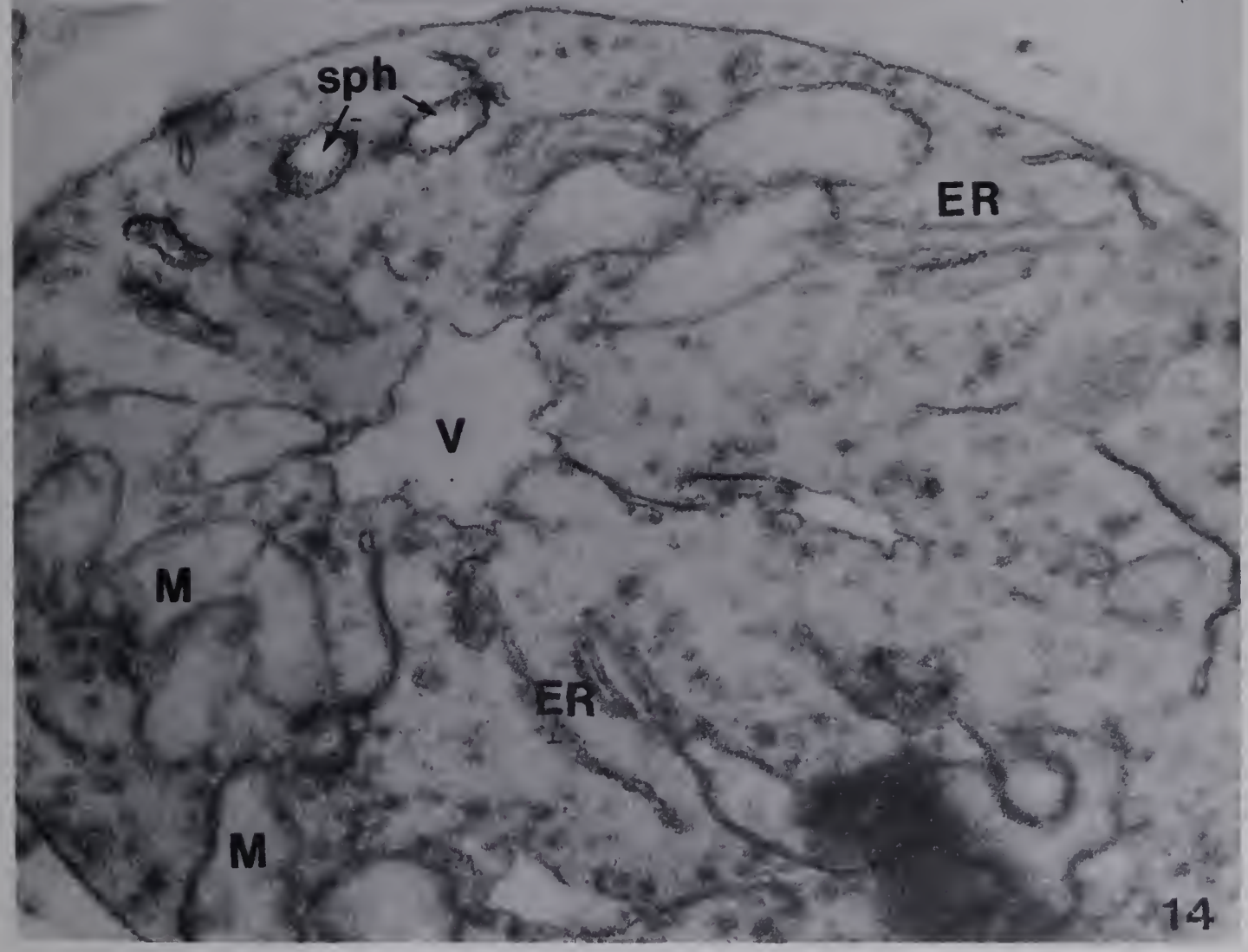
$KMnO_4$ - fixed lead citrate - stained.

Figure 13 X 49,000

Figure 14 X 76,000



13



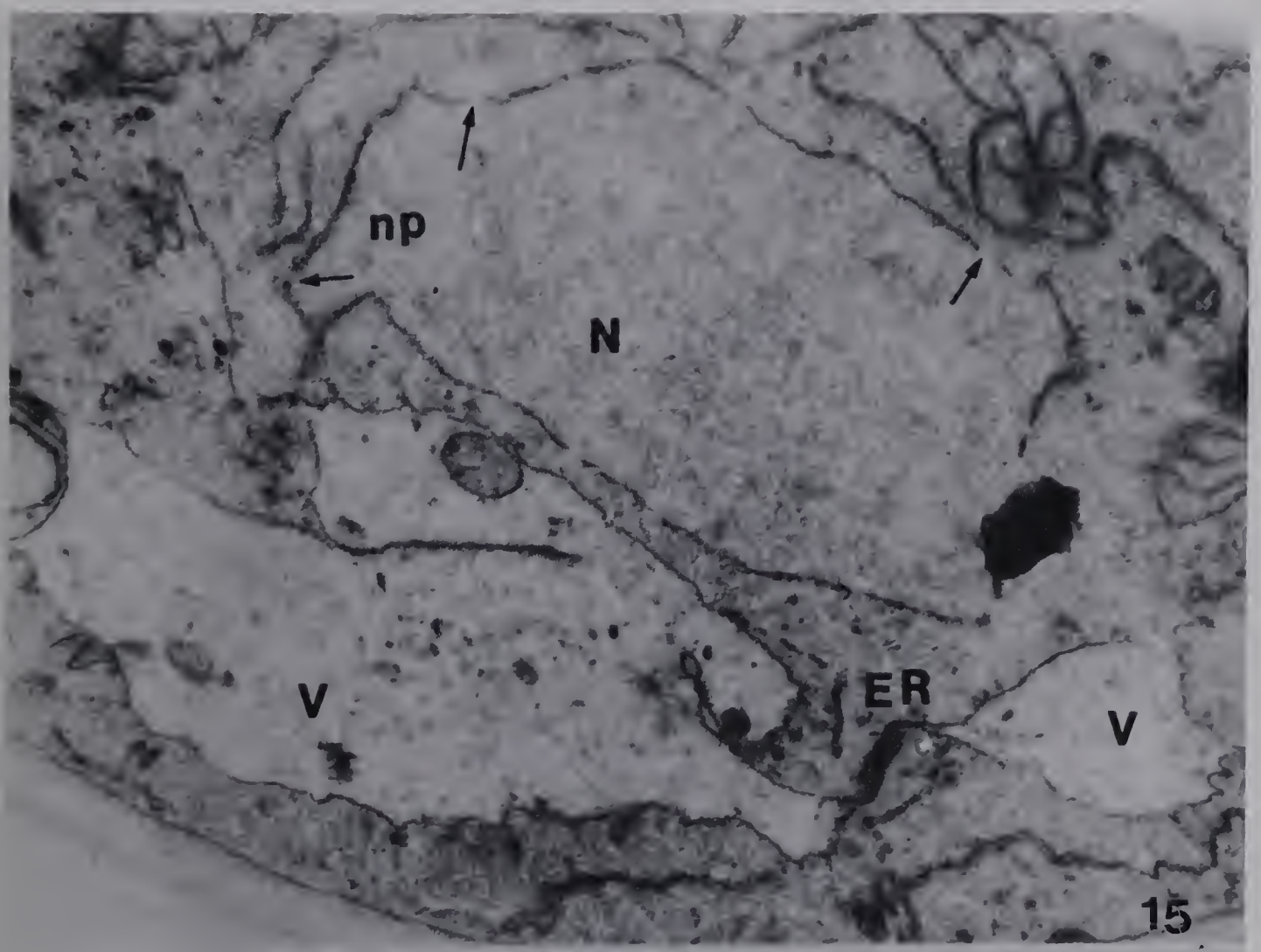
14

Figures 15 and 16. Longitudinal sections of germinated conidia fixed in $KMnO_4$ and stained in lead citrate illustrative of the role endoplasmic reticulum (ER) may play in the formation of vacuoles (V).

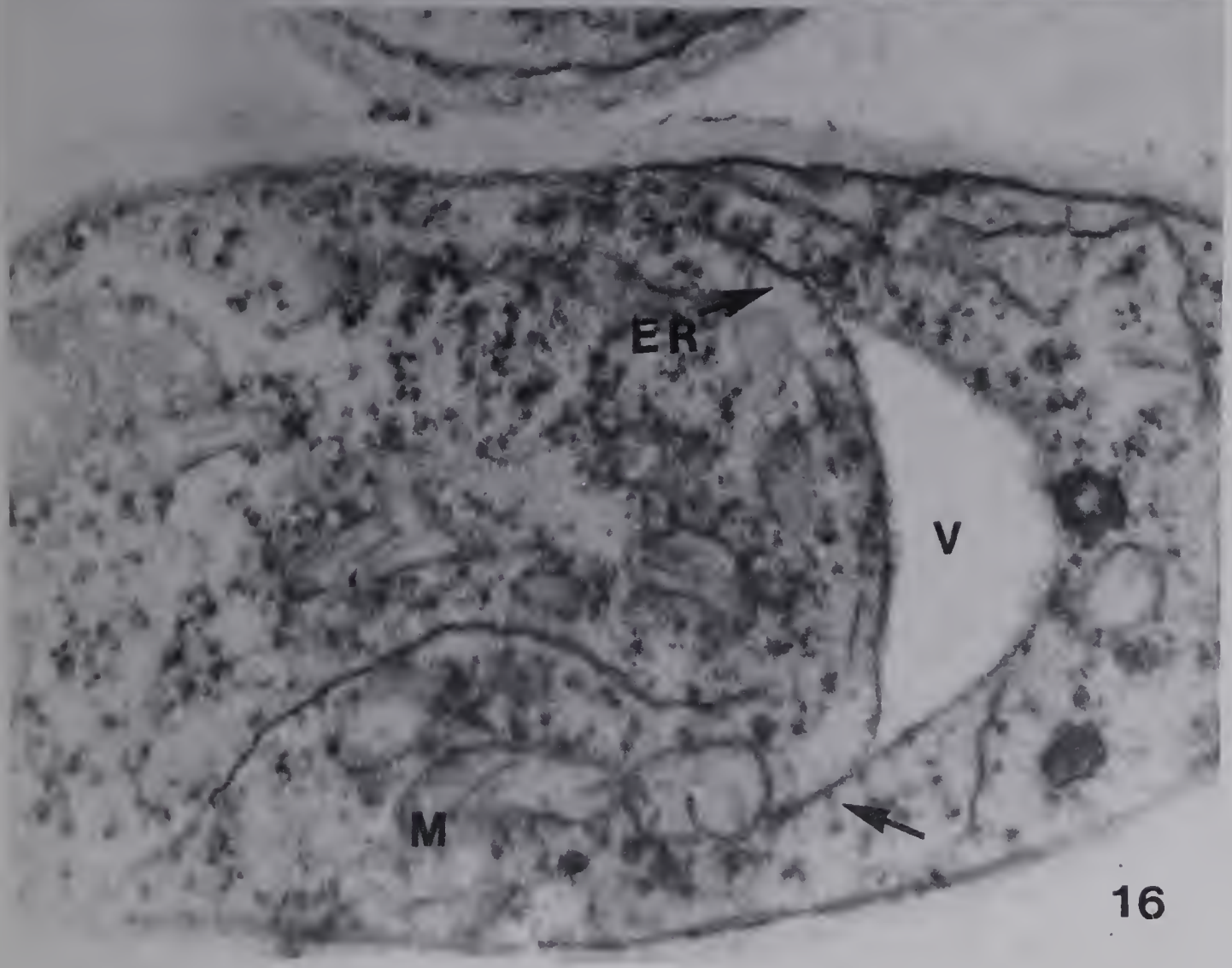
In addition Figure 15 shows several nuclear pores (np) in a rather elongated nucleus (N)

Figure 15 X 52,500

Figure 16 X 55,500



15

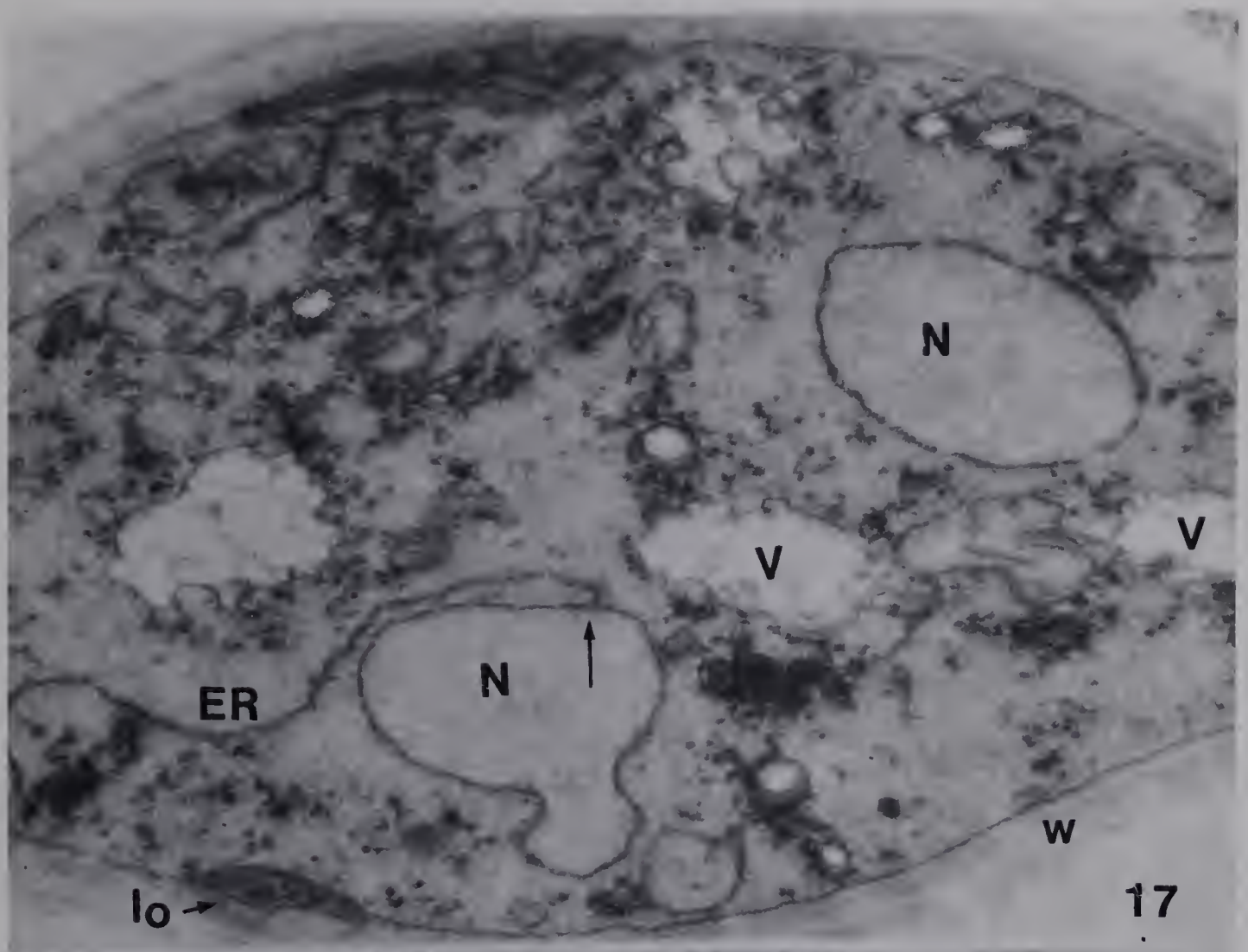


16

Figures 17 and 18. $KMnO_4$ - fixed and lead citrate-stained longitudinal sections of germinated conidia of N. crassa illustrating the continuity which exists between the nucleus (N) and endoplasmic reticulum (ER). In Figure 17 a continuity between the outer nuclear membrane and the ER (arrow) is seen. In Fig. 18 the arrow indicates a similar continuity.

Figure 17 X 38,500

Figure 18 X 62,000



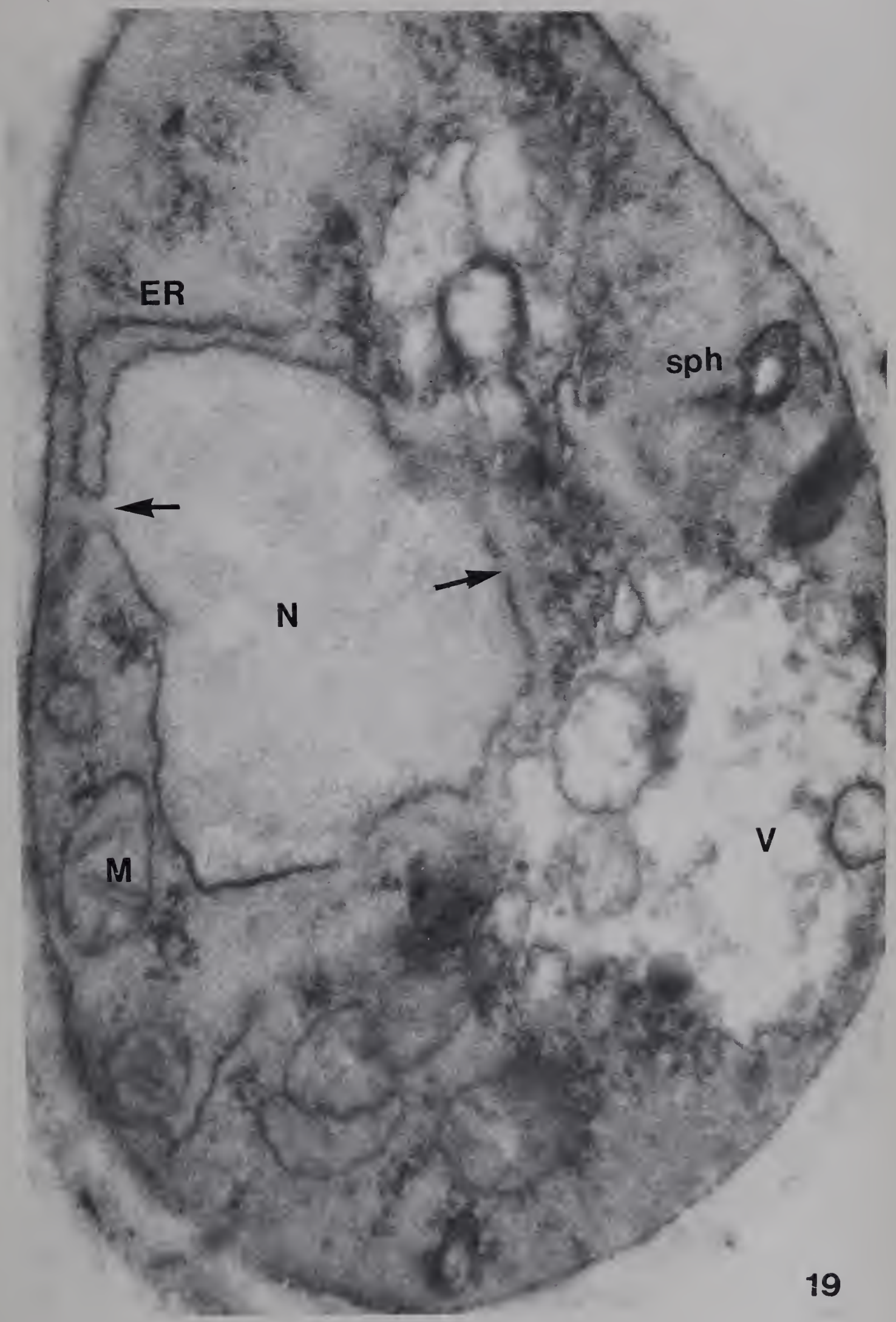
17

18

Figure 19. Longitudinal section of a germinating conidium fixed in $KMnO_4$ and stained with lead citrate illustrates the continuity of the nucleus (N) with the endoplasmic reticulum (ER).

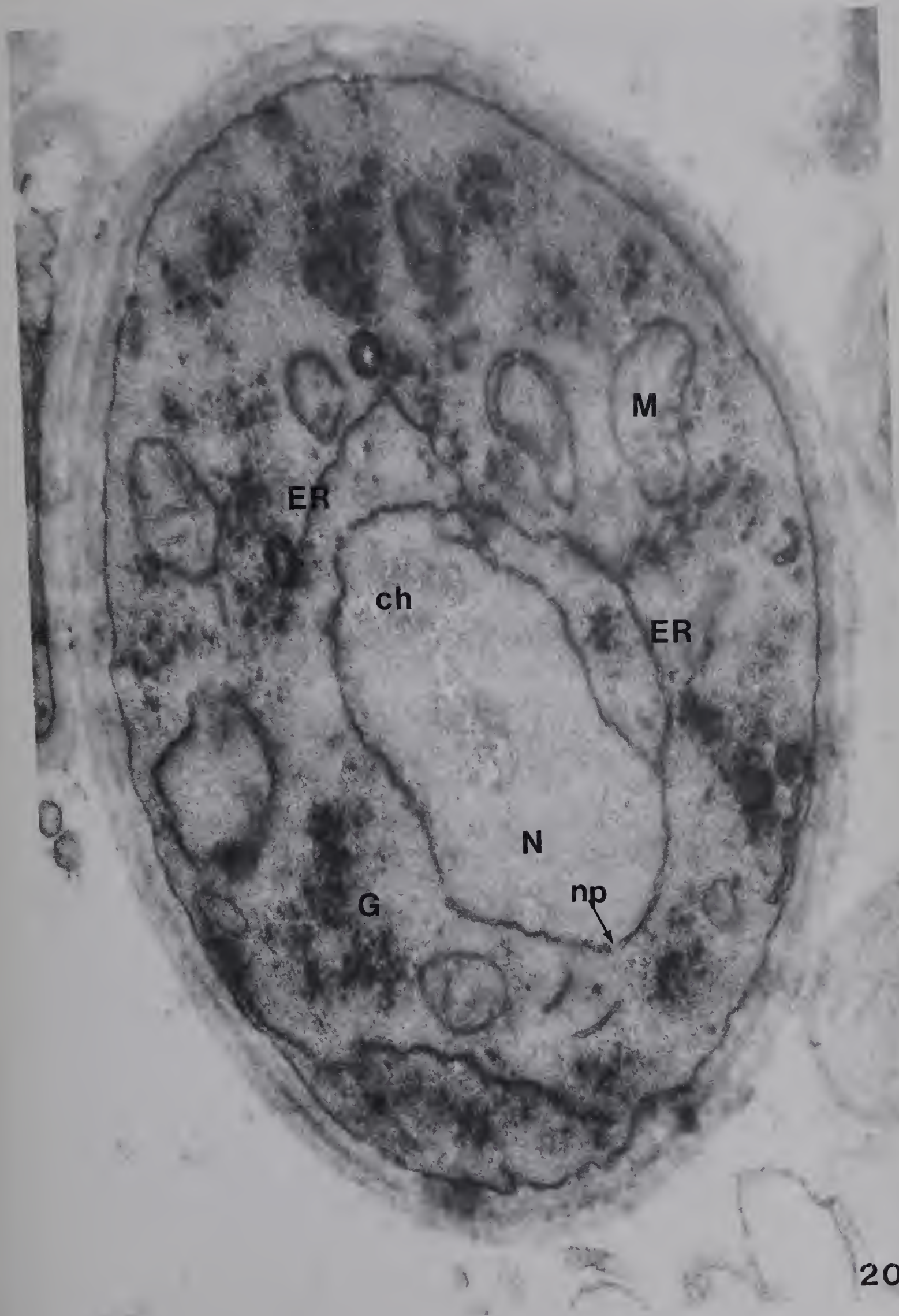
The arrow points to a "pore" which may allow a ready exchange of nuclear and cytoplasmic constituents.

X 33,500



19

Figure 20. $KMnO_4$ - fixed and lead citrate-stained conidium illustrating the continuity of the nuclear envelope with the ER (arrow). X 66,000

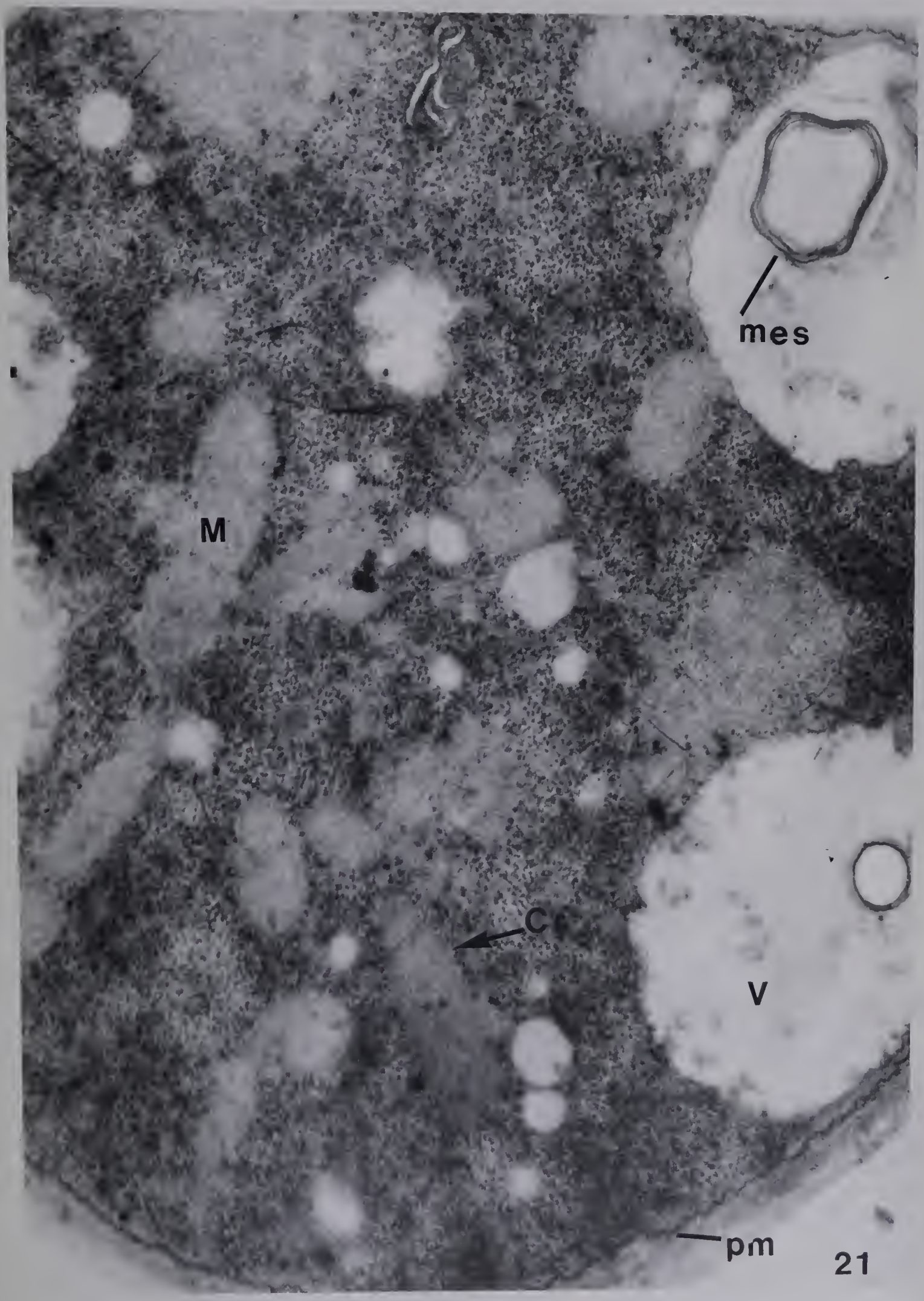


20

Figure 21 and 22. Serial sections of a conidium fixed in glutaraldehyde and osmium tetroxide showing membraneless crystalline inclusions free in the cytoplasmic matrix (Cr). Numerous alpha particles (G) are seen in Figure 21. Both micrographs contain a cytolysome containing myelinoid-like mesosomes. In addition, the cytolysome in Figure 22 contains numerous unstructured electron-dense particles while the one in Fig. 22 contains what appears to be a portion of cytoplasm containing glycogen (G).

X 49,000

X 70,000



21

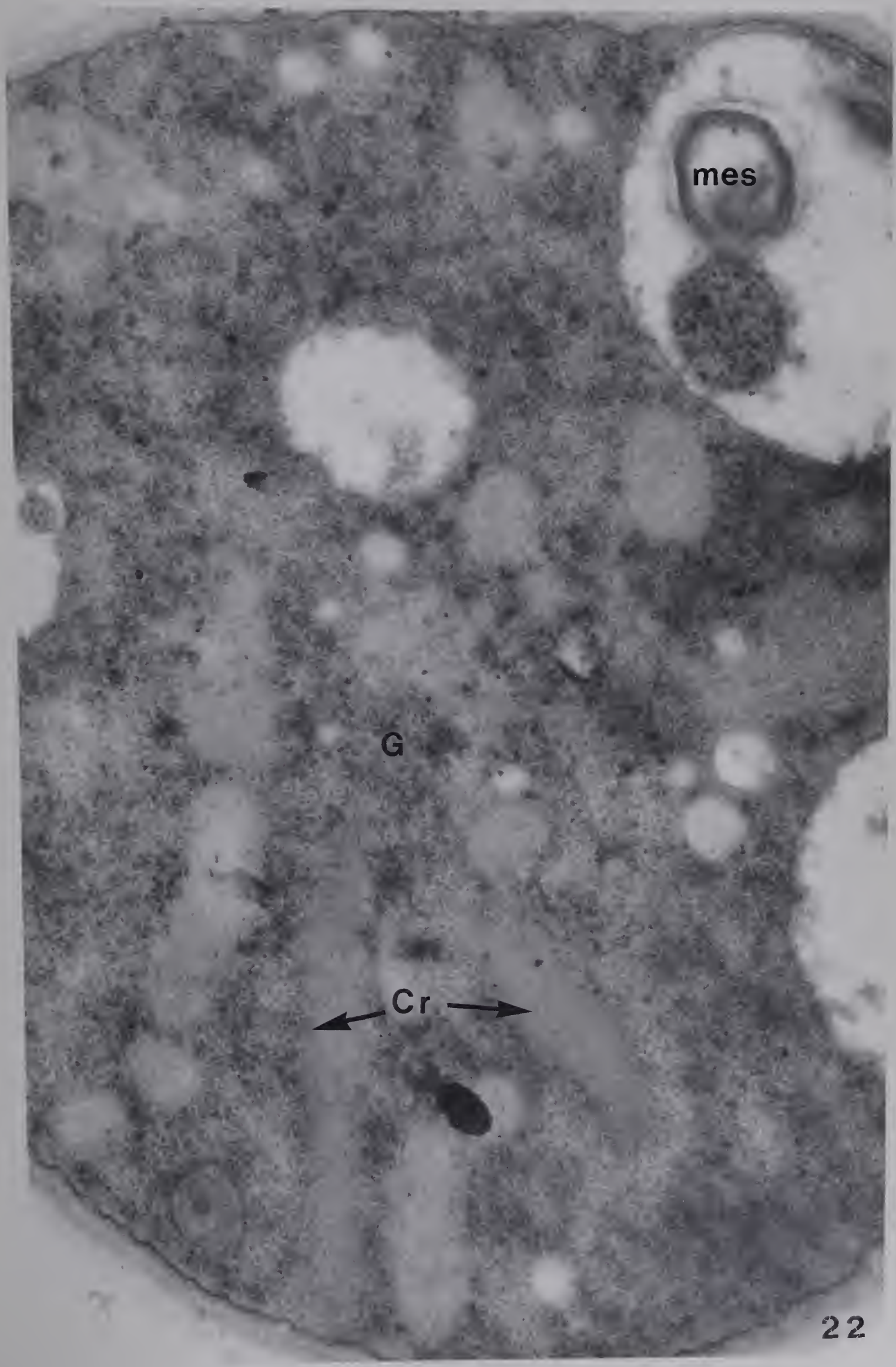

mes
G
Cr

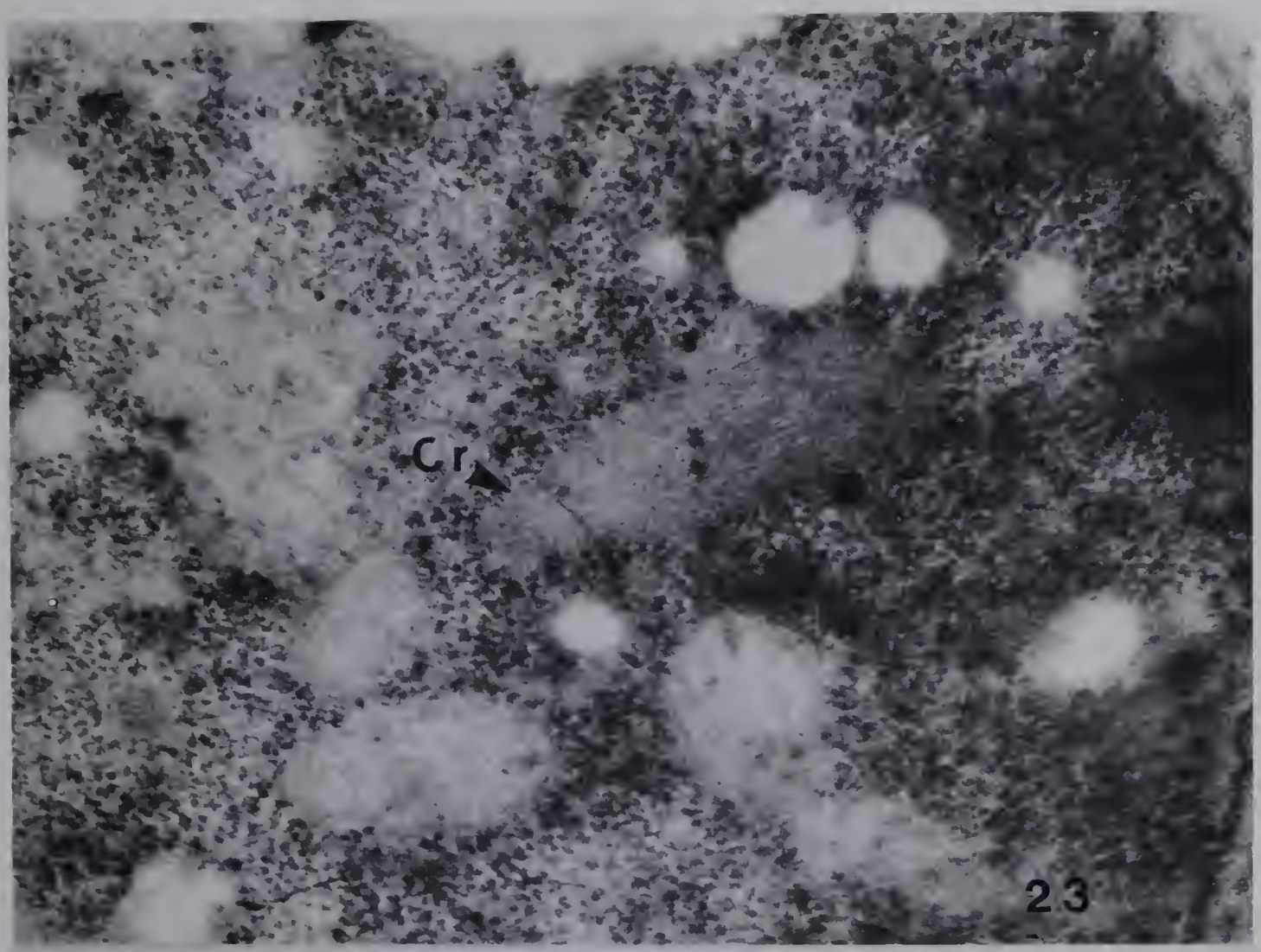
Figure 23. An enlarged portion of a germinating conidium showing longitudinal view of a crystalline inclusion (Cr), consisting of electron opaque structures arranged in a parallel array. The structure does not appear to be membrane-bound.

X 48,600

Figure 24. Transverse section showing a hexagonally packed pattern. A surrounding membrane is not apparent.

X 135,000

Figure 23 and 24 fixed in glutaraldehyde and osmium tetroxide and stained in uranyl acetate and lead citrate.


Cr
23

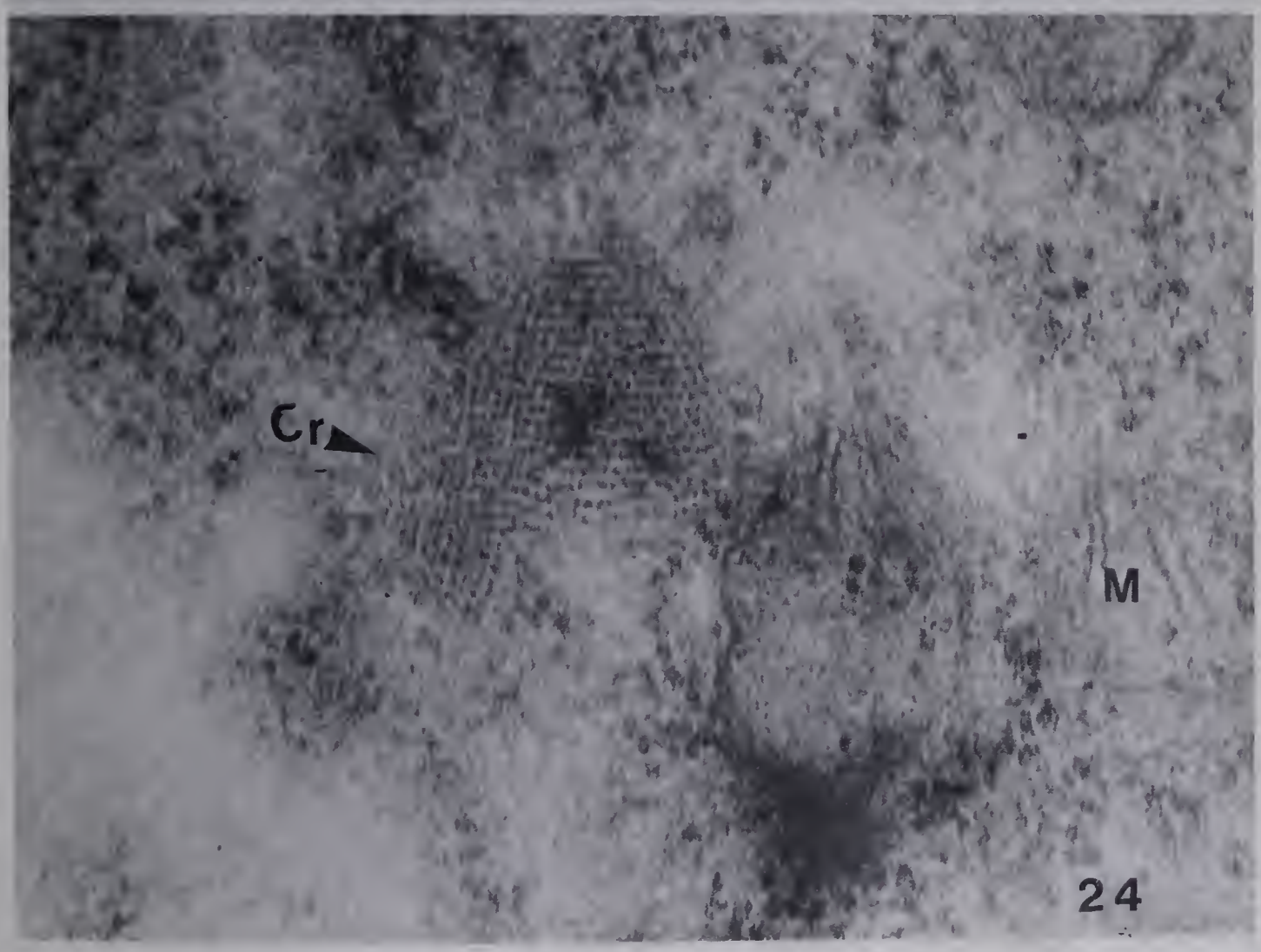
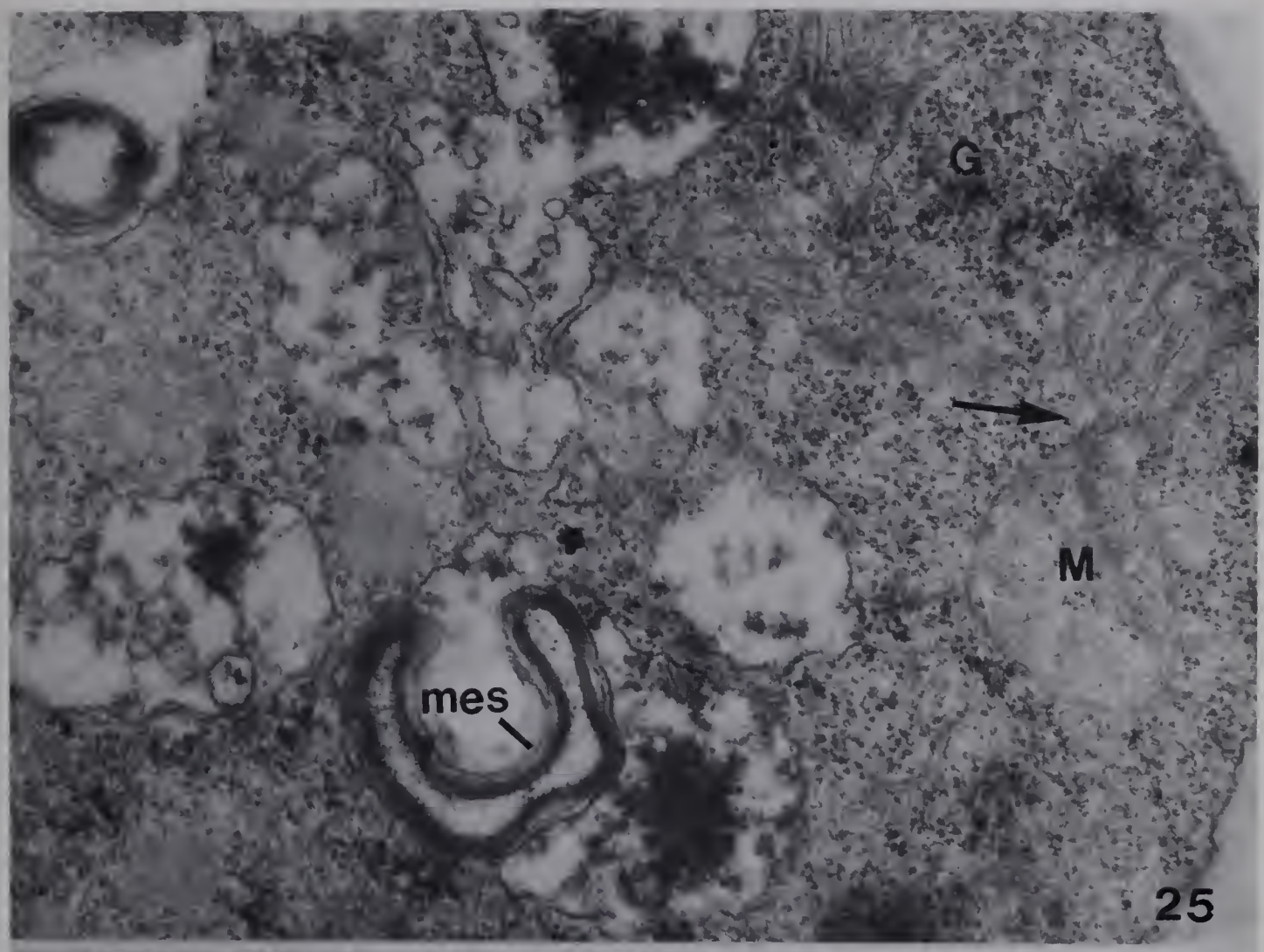

Cr
M
24

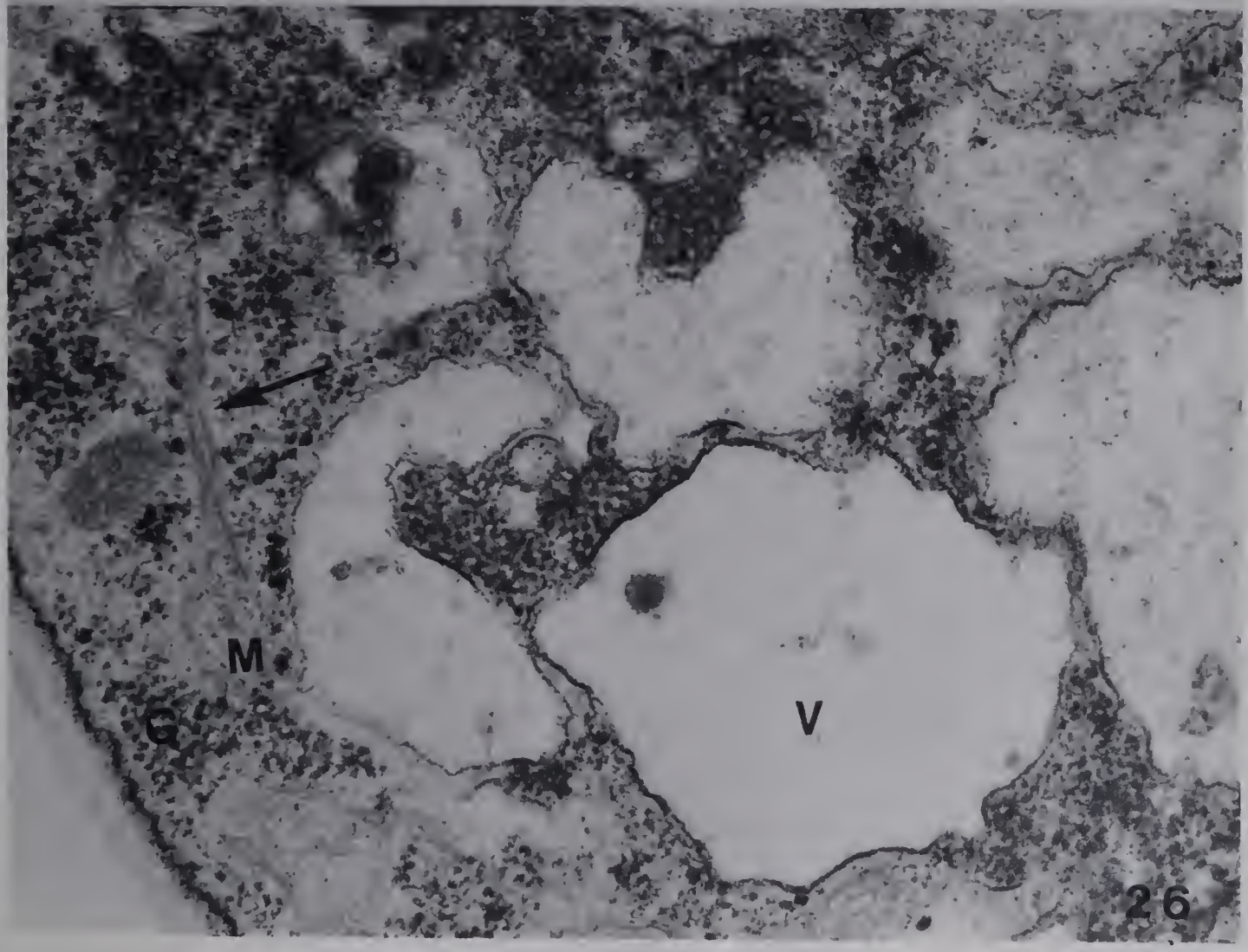
Figure 25 and 26. Transverse sections of germinated conidia illustrating mitochondria (M) undergoing division (arrow). Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate and lead citrate-stained.

Figure 25 X 90,800

Figure 26 X 81,700



25

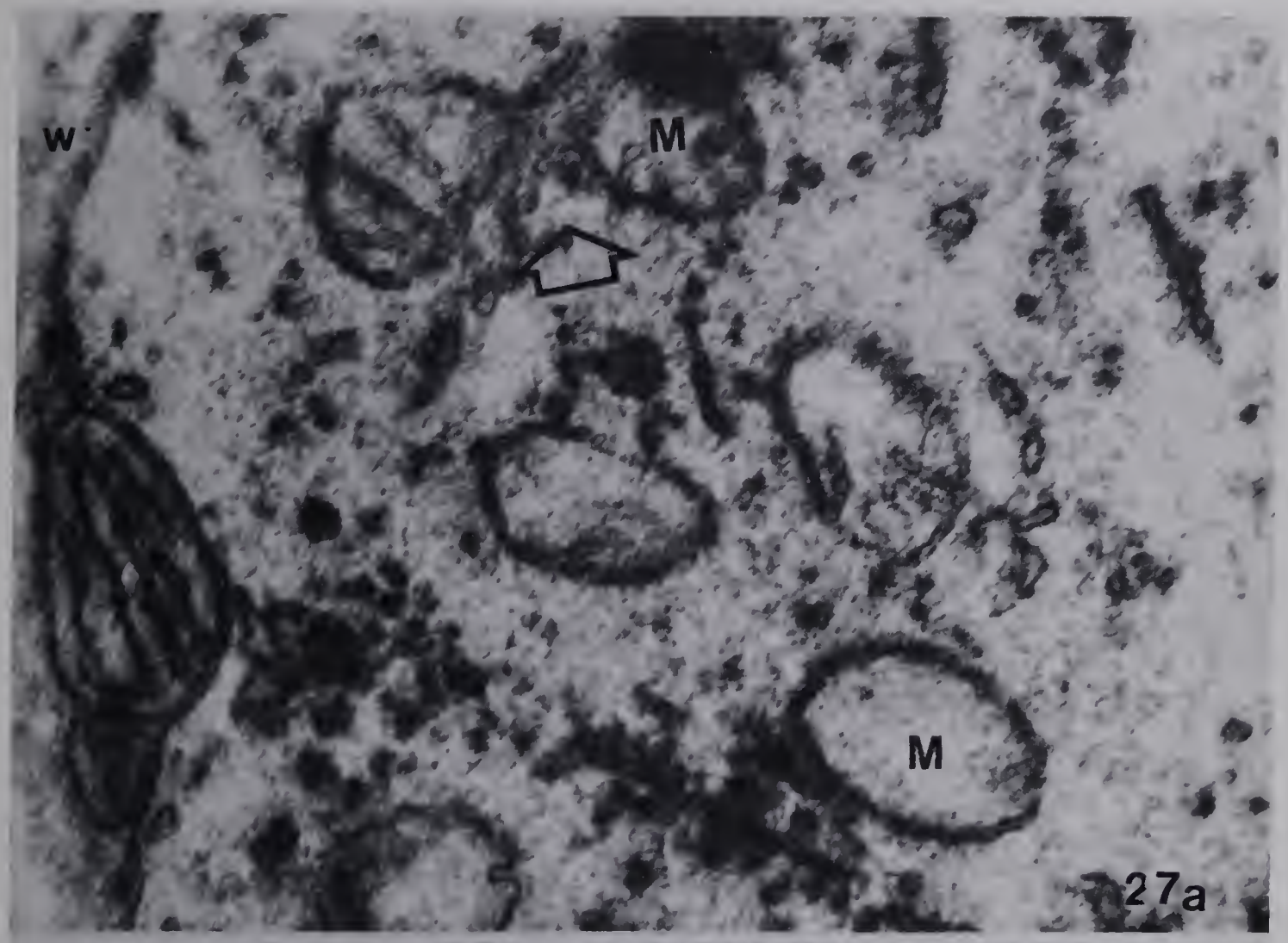


26

Figure 27 a and 27 b. Transverse serial sections of a conidium fixed in $KMnO_4$ and stained with lead citrate. The upper micrograph shows a mitochondrion (M) in the process of dividing. The lower micrograph shows these separated.

Figure 27 a X 110,800

Figure 27 b X 109,500


W
M
M
27a

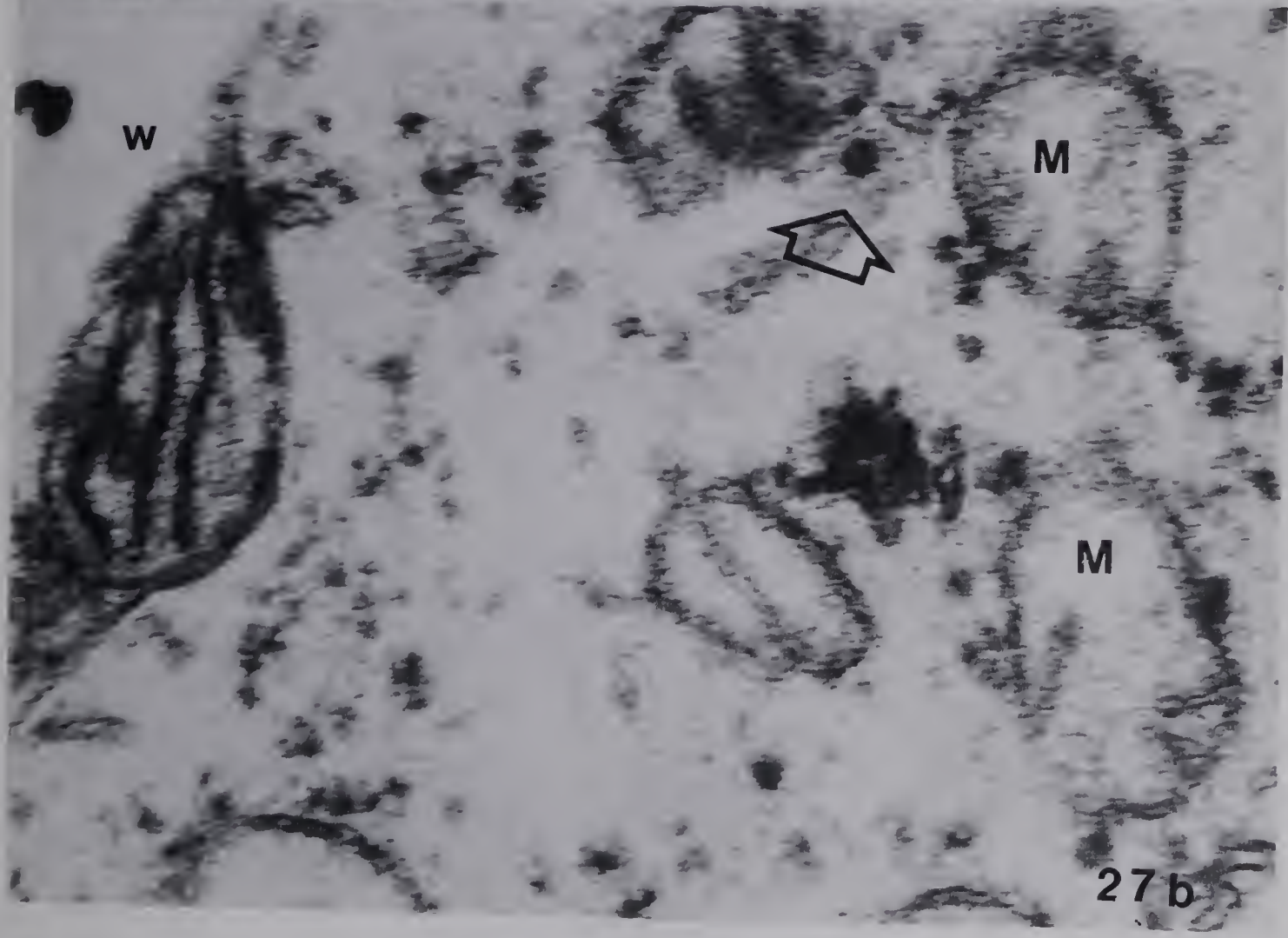
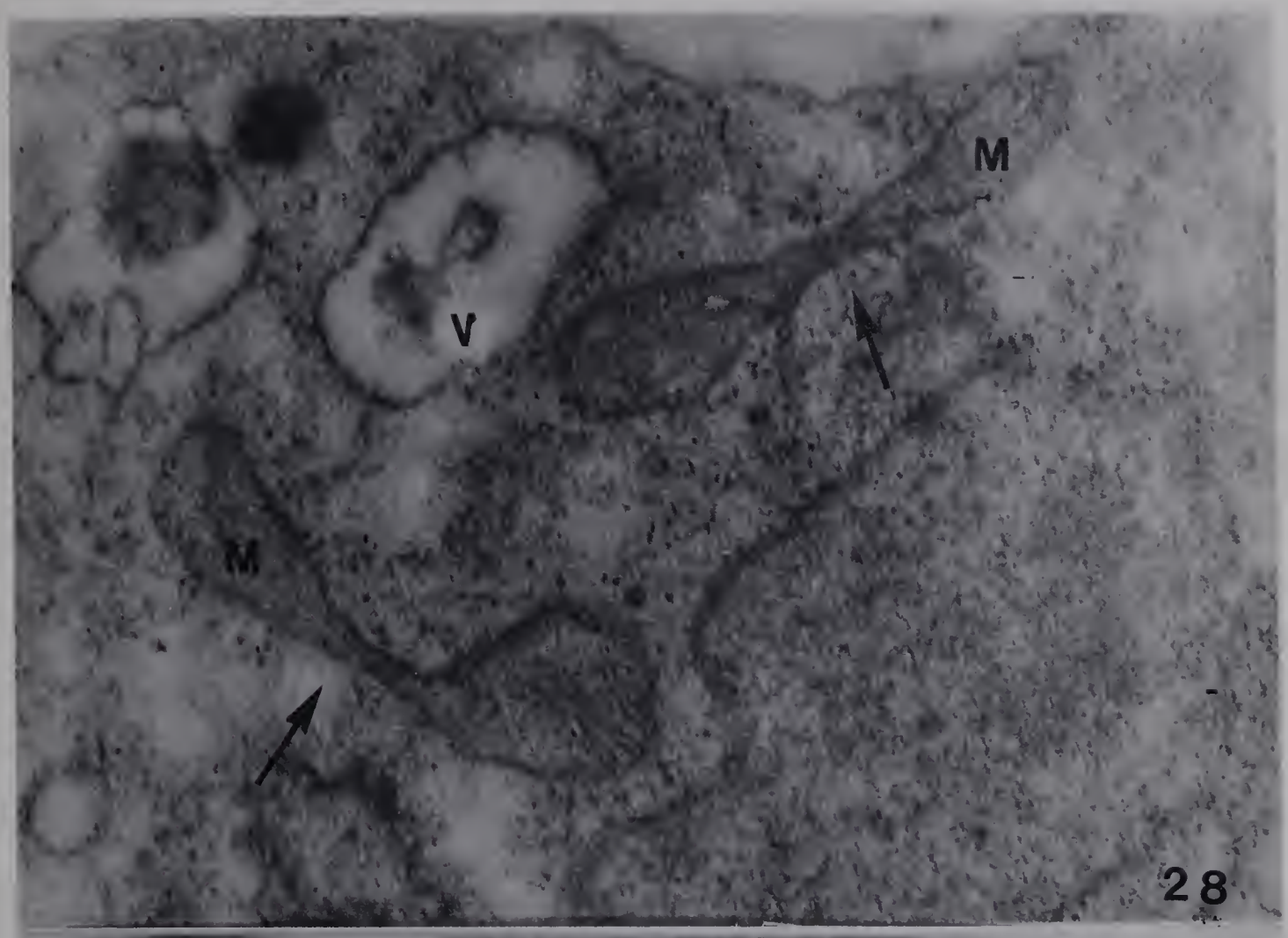

W
M
M
27b

Figure 28. An enlarged portion of a germinated conidium showing mitochondria undergoing division (arrows). Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate and lead citrate-stained.

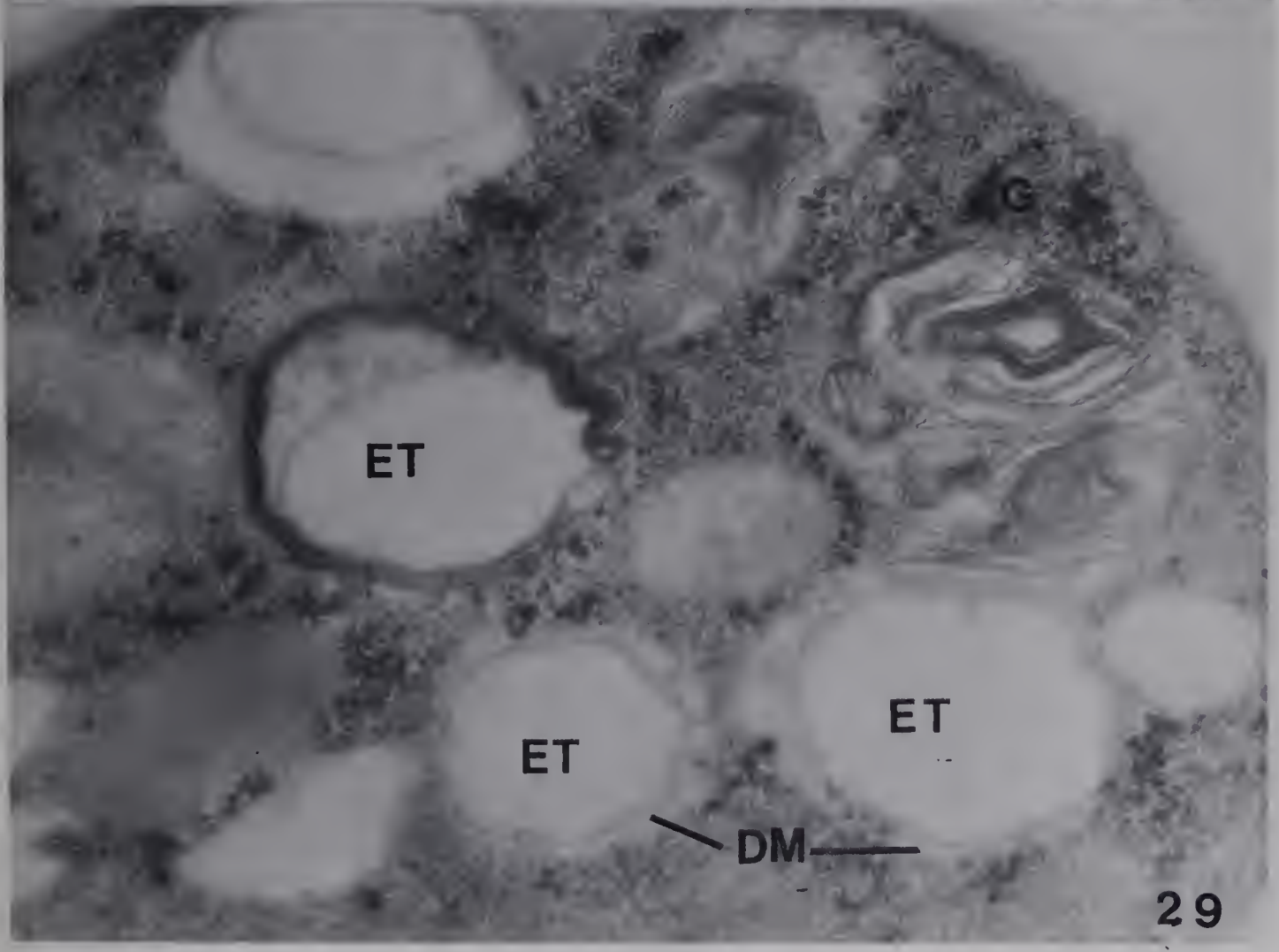
X 71,300

Figure 29. An electron micrograph of electron transparent (ET) areas frequently found in the conidia. The electron transparent areas shown in the micrograph are bounded by a double membrane. Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate and lead citrate-stained.

X 49,200



28

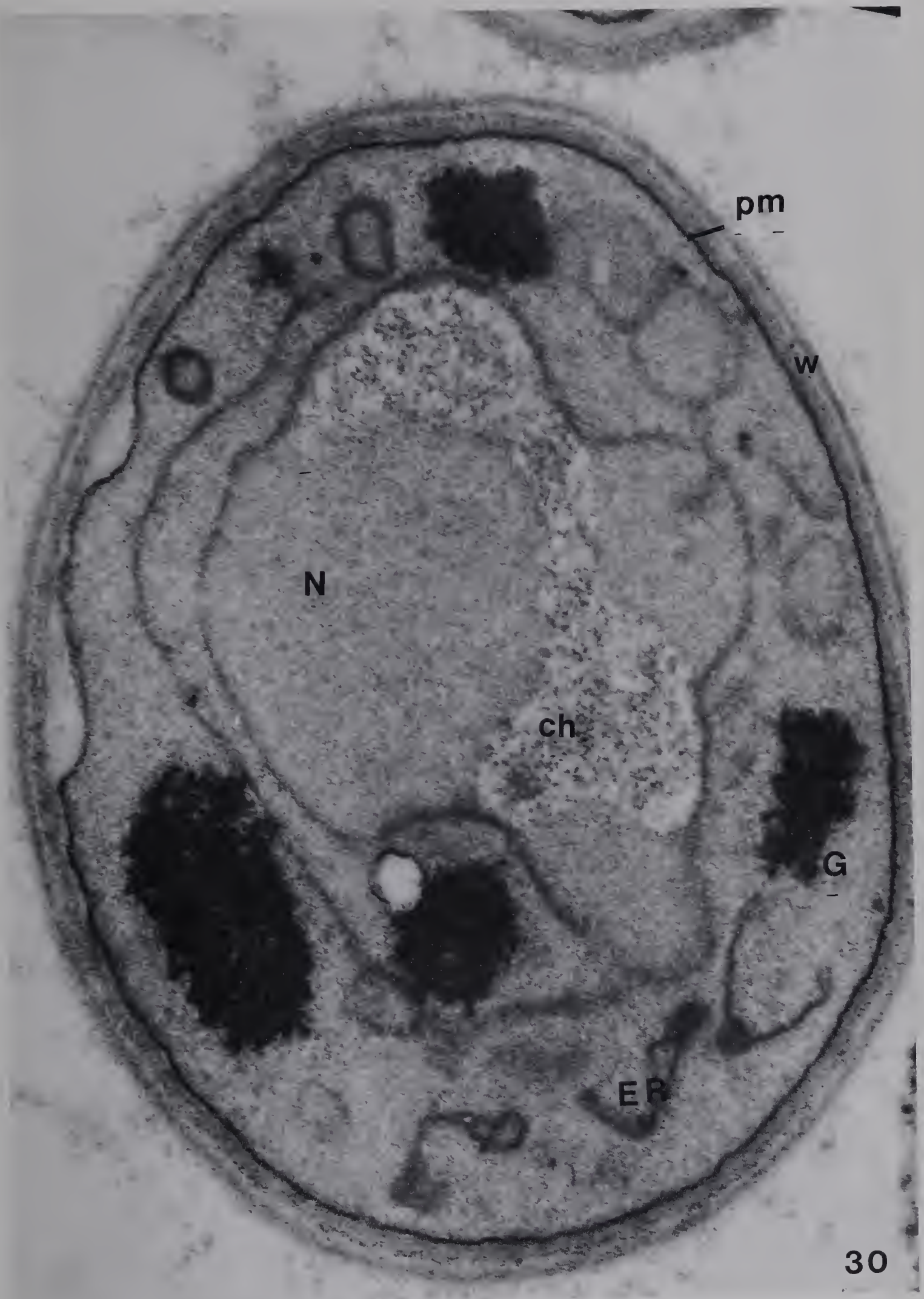


29

Figure 30. Transverse section of a germinated conidium illustrating aggregations of glycogen (G) commonly referred to as rosette particles. The nucleus (N) contains darkly stained chromatic (ch) areas. Endoplasmic reticulum (ER) in the lower portion of the photograph illustrates a reticular formation which has dilated itself into a terminal bulb.

$KMnO_4$ fixed lead citrate stained

34,000



30

Figure 31. Transverse section of germinated conidia of N. crassa fixed in glutaraldehyde and osmium tetroxide and stained in uranyl acetate and lead citrate showing a mitrochondrion in the process of division and numerous microtubules (arrows).

X 67,500

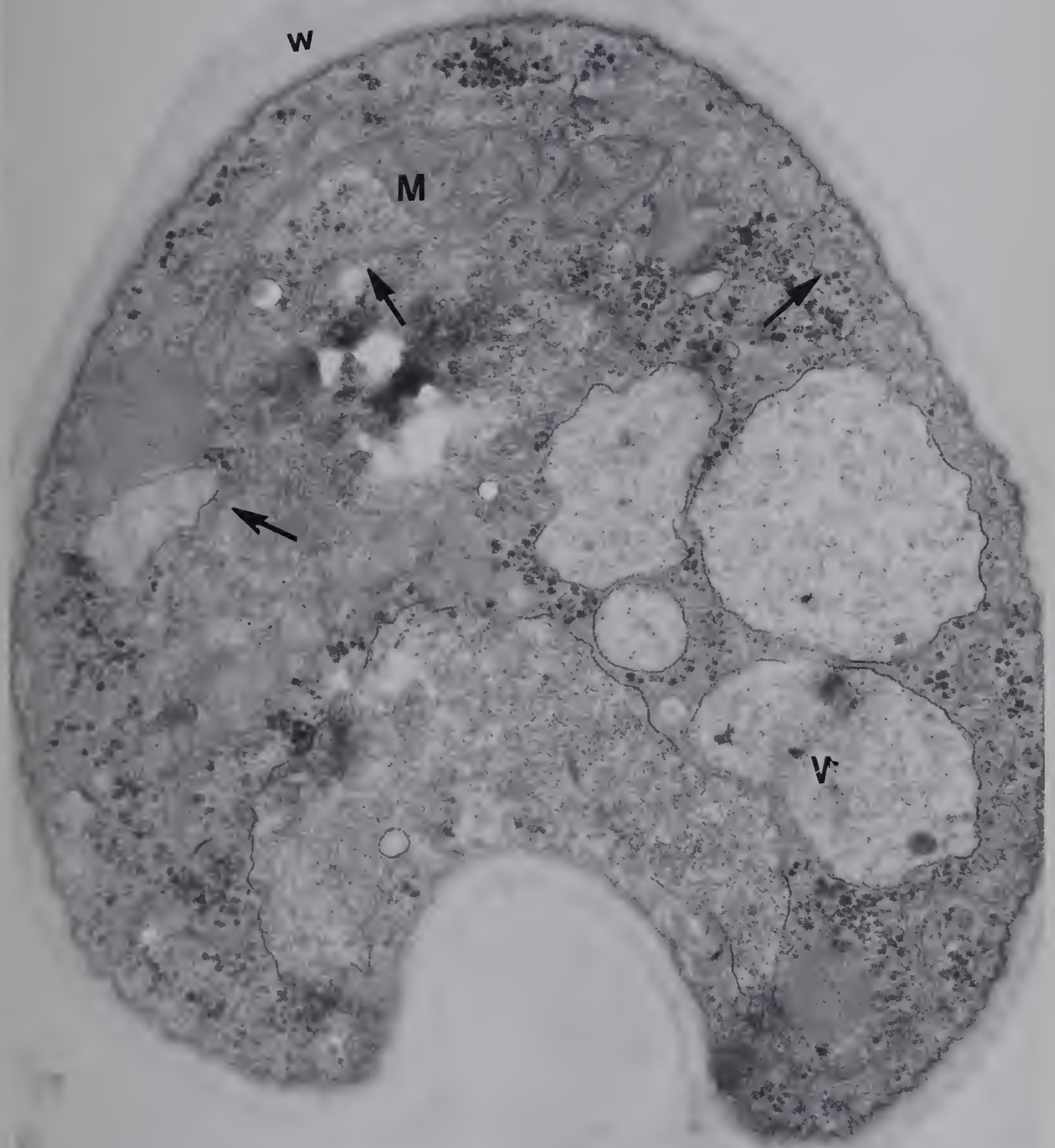
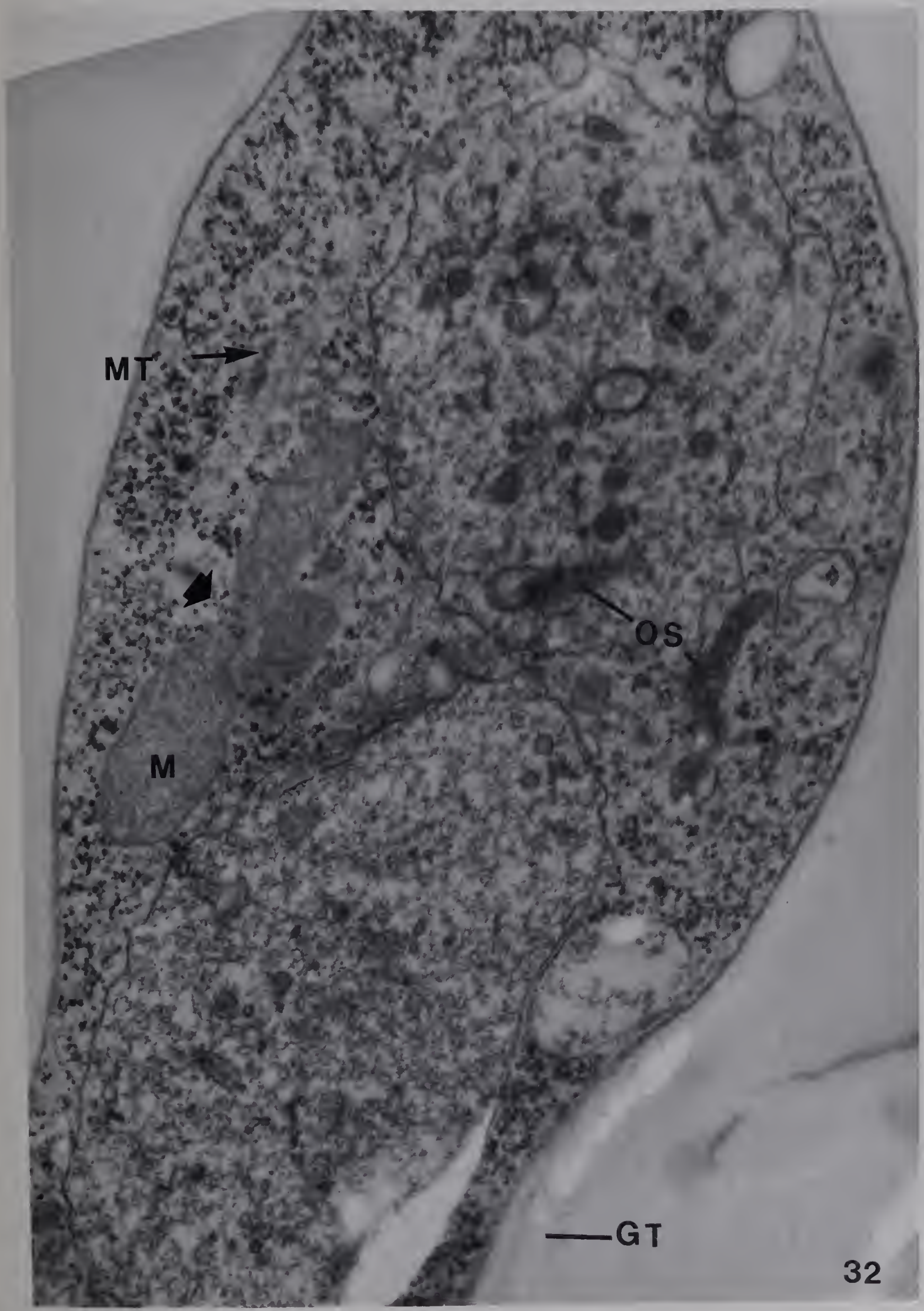

W
M
V

Figure 32. A longitudinal section of a germinating conidium showing a mitochondrion probably in the process of division (M) as well as a number of microtubuli (MT).

Section fixed in glutaraldehyde and osmium tetroxide and stained in uranyl acetate and lead citrate.

X 74,500



32

Figure 33. An enlarged view of a germinated conidium showing numerous osmiophilic bodies, the composition of which has not been unequevocally proven. On the basis of their staining reaction they are considered to be lipid. Numerous bizarrely shaped masses of lipids appear in the cyloplasmic matrix (OS1 OS2 OS3) as well as several electron transparent areas (ET) are also shown. Arrows indicate microtubules.

X 45,000

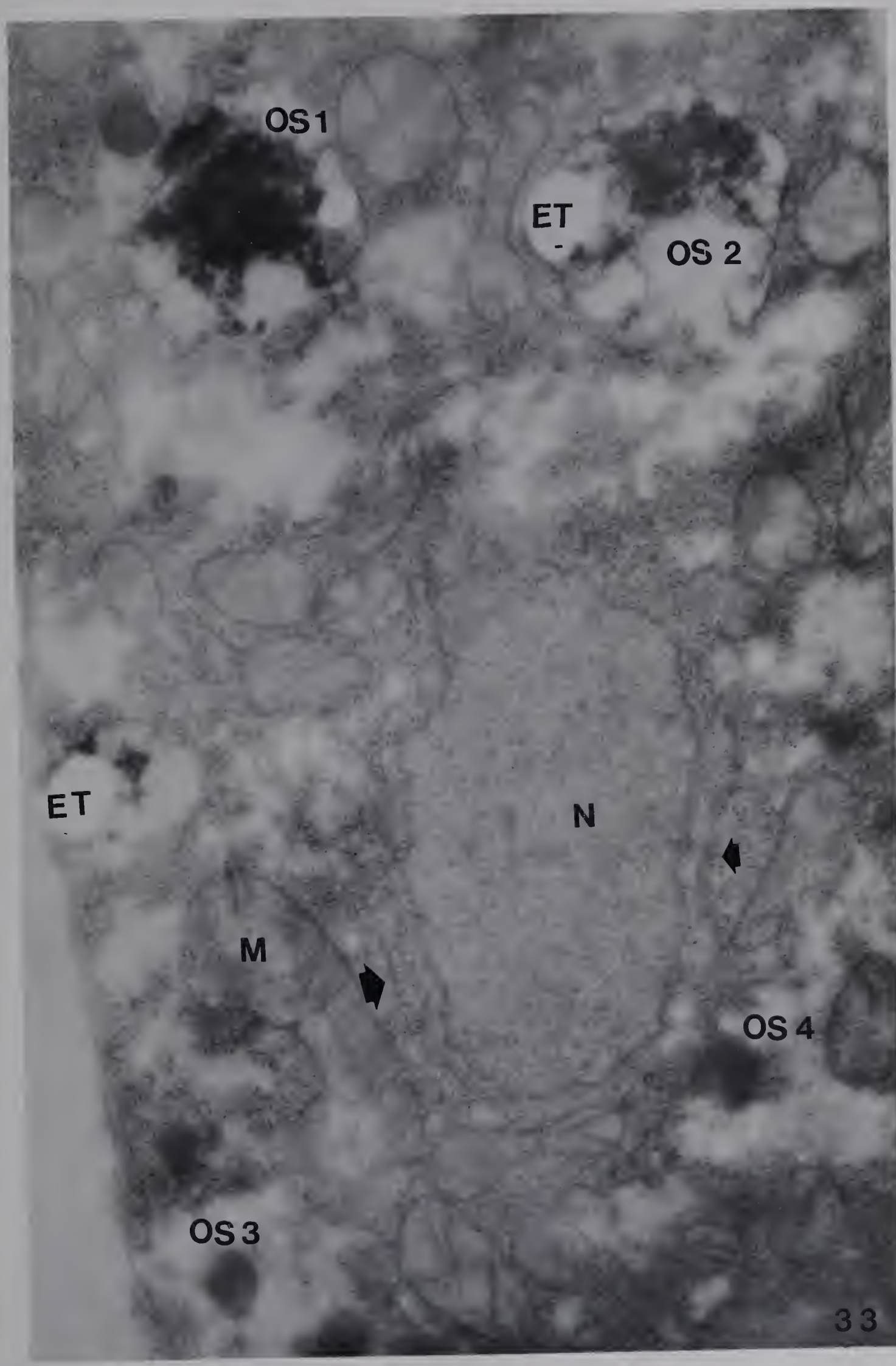
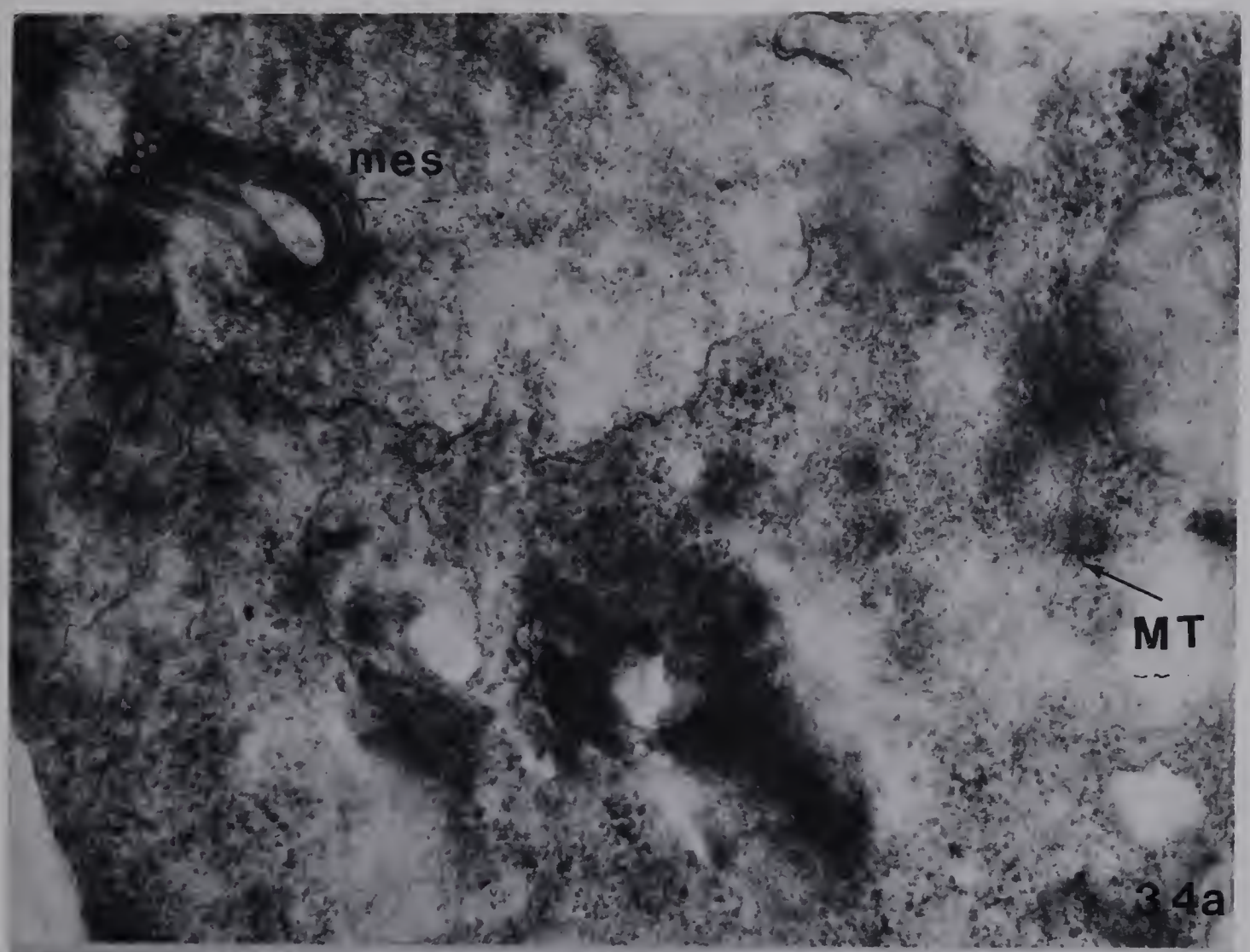

OS 1
ET
OS 2
ET
N
M
OS 4
OS 3
33

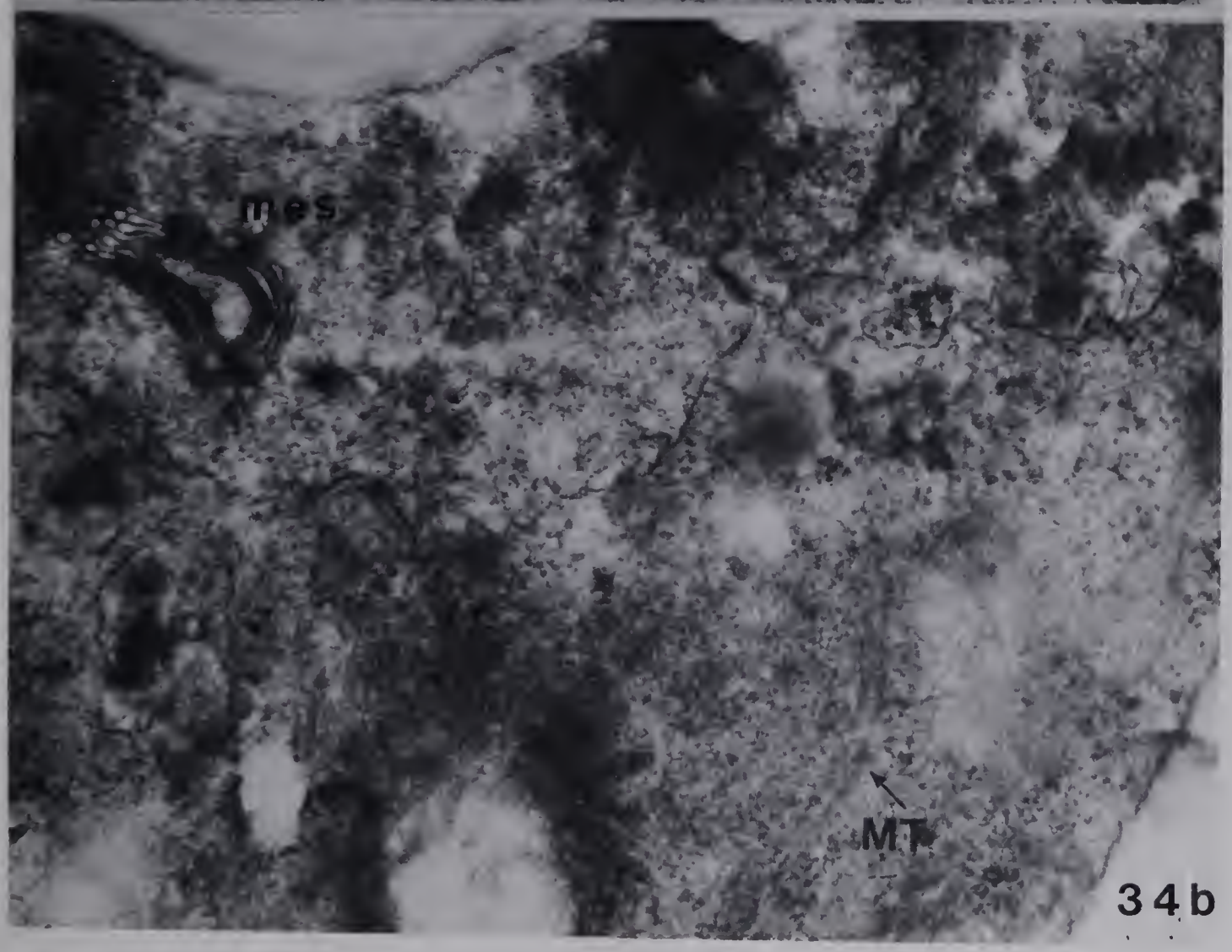
Figure 34a and 34b Longitudinal serial sections of a germinated conidium of N. crassa fixed in glutaraldehyde and osmium tetroxide and stained with uranyl acetate and lead citrate showing mesosomes (mes) and microtubles (MT)

Figures 34a and 34b

X 73,600



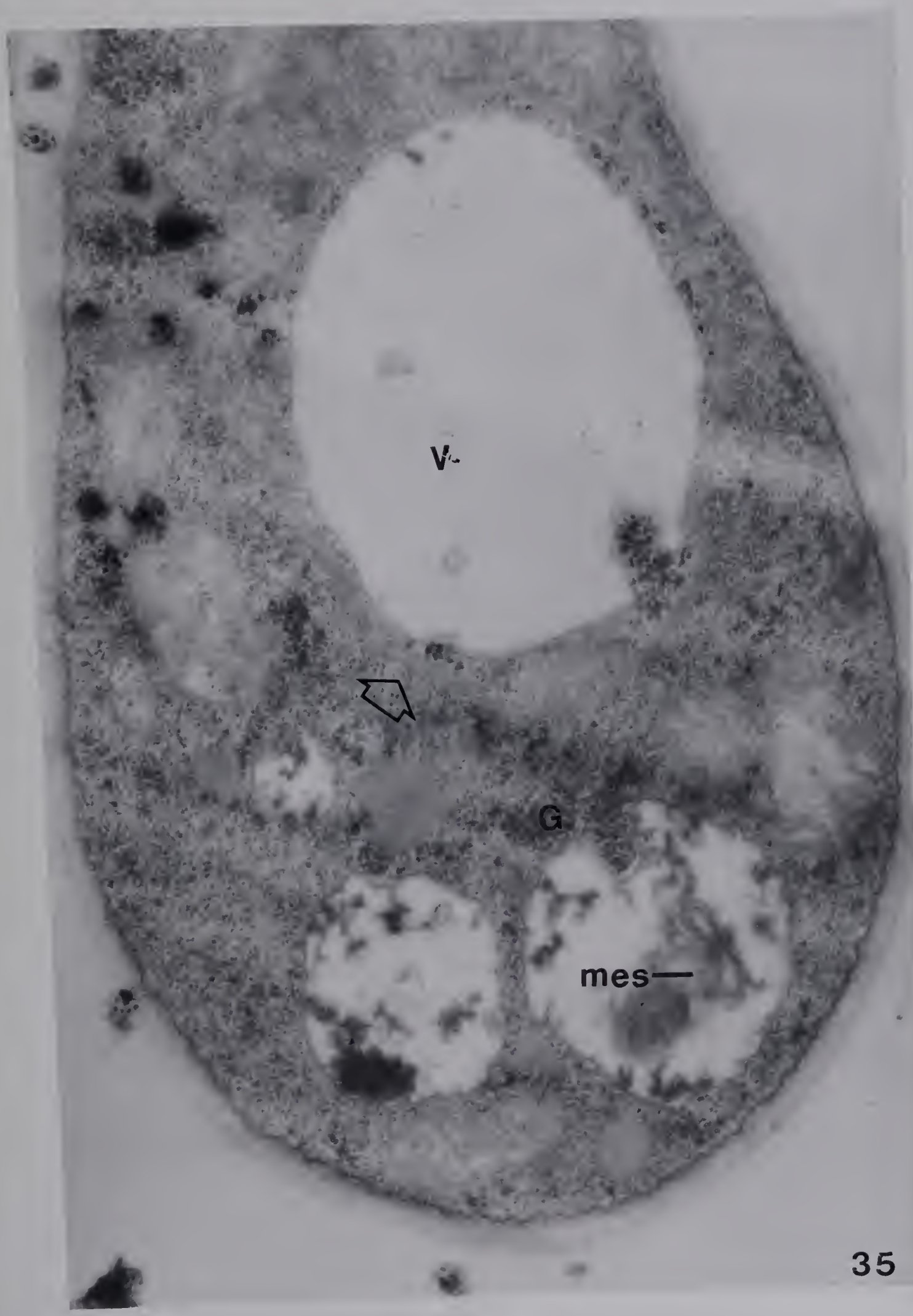
34a



34b

Figure 35. A germinating conidium of N. crassa illustrating the microtubuli (arrow) frequently encountered near the periphery of vacuoles (V). Also seen is a membrane-bound vacuole containing a mesosome (mes). Section fixed in glutaraldehyde and osmium tetroxide and stained in uranyl acetate and lead citrate.

X 45,000



35

Figure 36. An enlarged serial section of Figure 35 showing microtubules (arrow) and the myelinoid-like mesosome (mes) within the confines of the vacuole. (V).

Section fixed in glutaraldehyde and osmium tetroxide and stained in uranyl acetate and lead citrate.

X 54,000

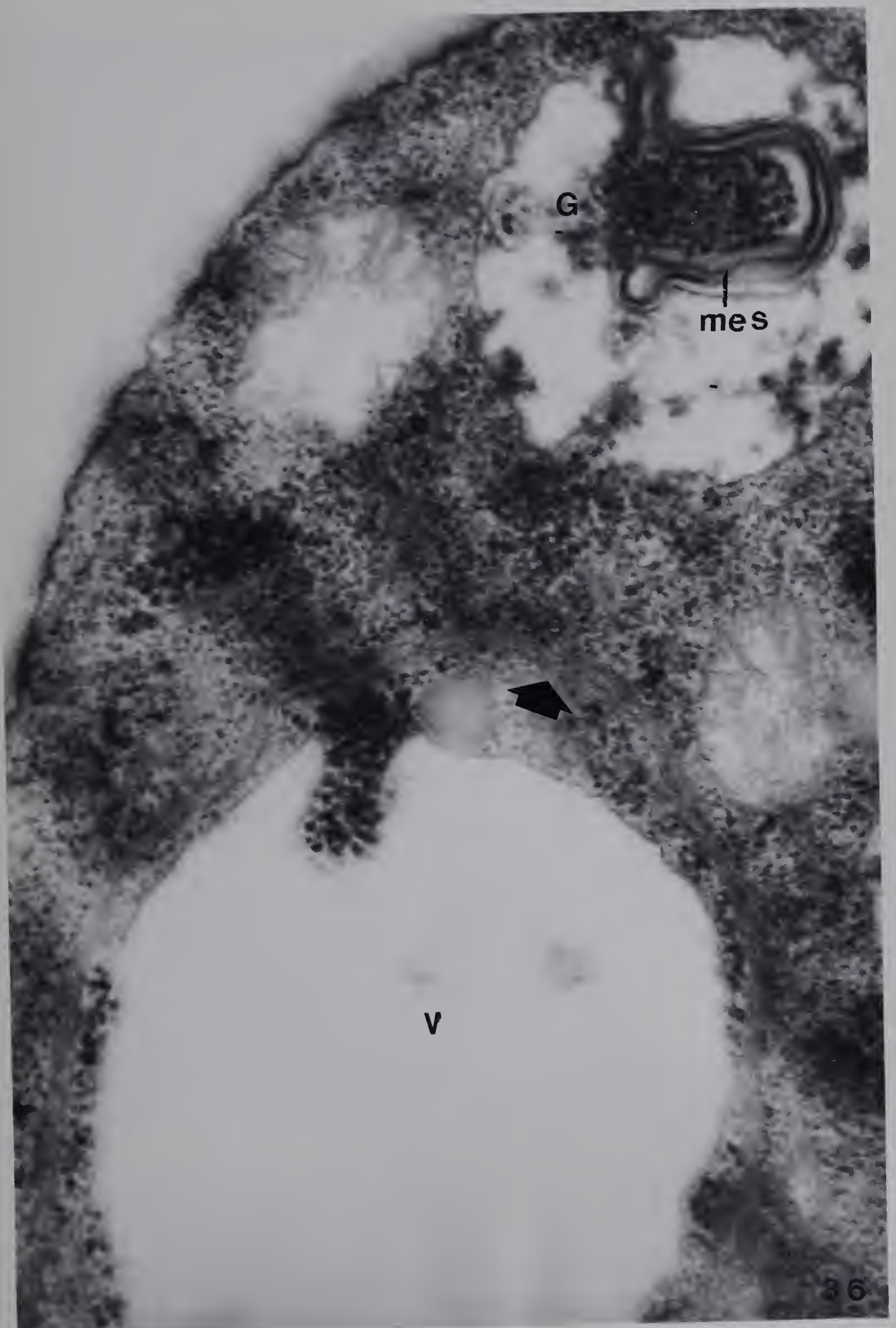

G
mes
V

Figure 37. A transverse section showing a large vacuole (V) containing mesosome-like configurations (mes) an osmiophilic body (OS) and various other electron-dense structures.

Note the microtubules (arrow) encircling the vacuole.

x 60,300

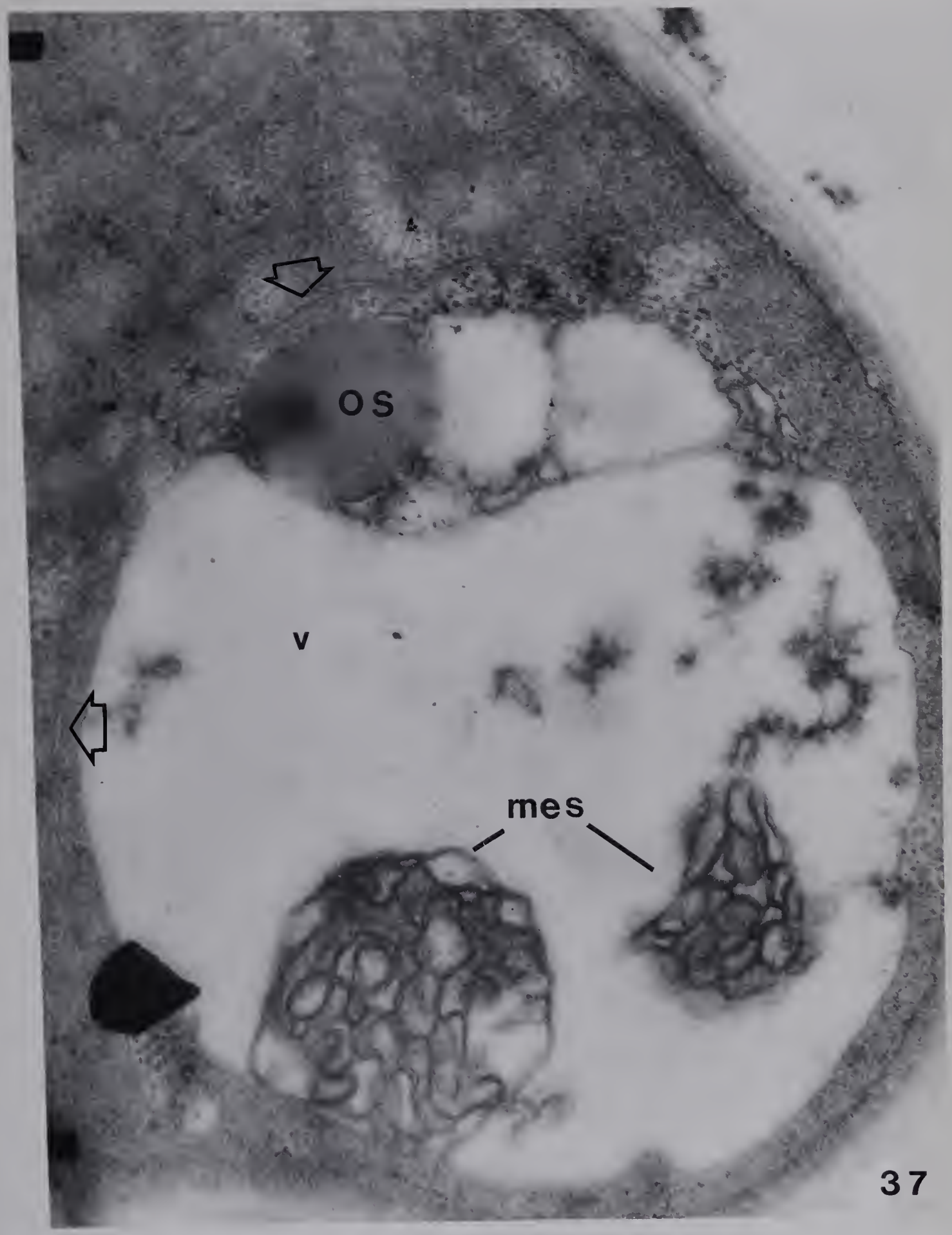

OS
V
mes

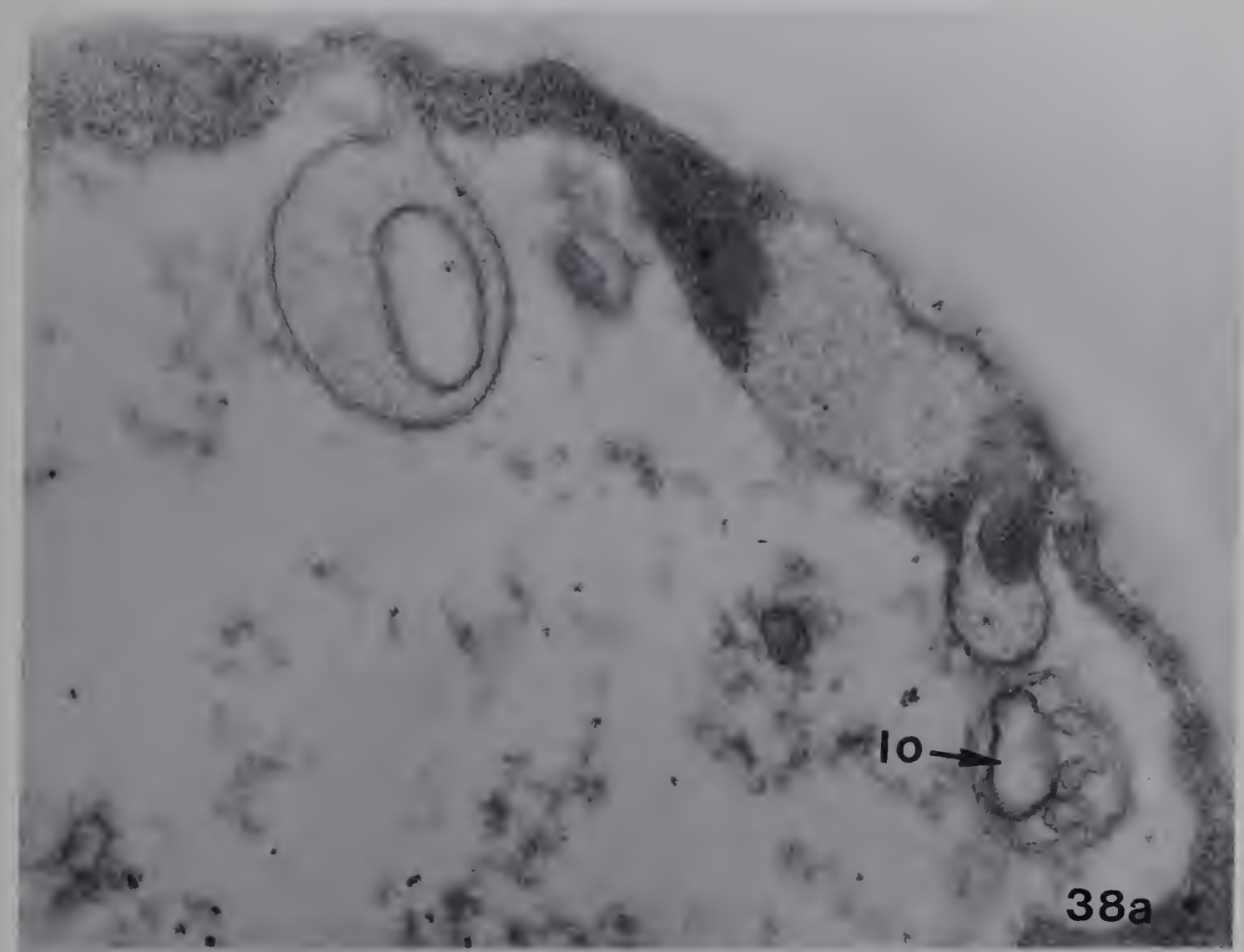
Figure 38 a. Transverse section of a vacuolating conidium fixed in glutaraldehyde and osmium tetroxide showing a lomasome (lo).

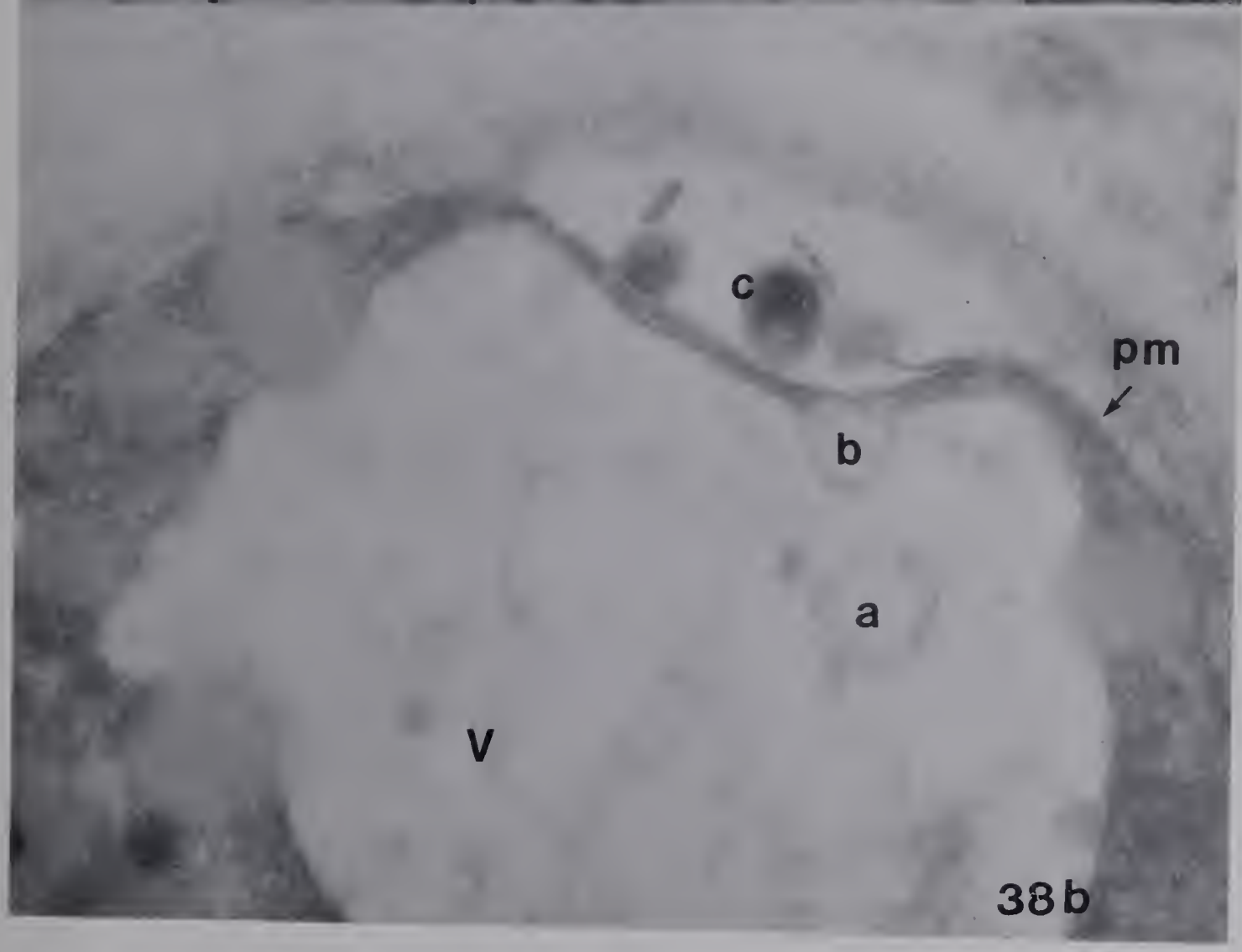
X 61,000

Figure 38b. Transverse section of a conidium of N. crassa fixed in glutaraldehyde and osmium tetroxide showing different but progressive functional levels of organization. (a) vesicle formed in the vacuole (V); (b) fusion of the lomasome with the plasma membrane (pm); (c) expelled secretory product.

X 60,000

Figs. 38a and 38b stained in uranyl acetate and lead citrate.

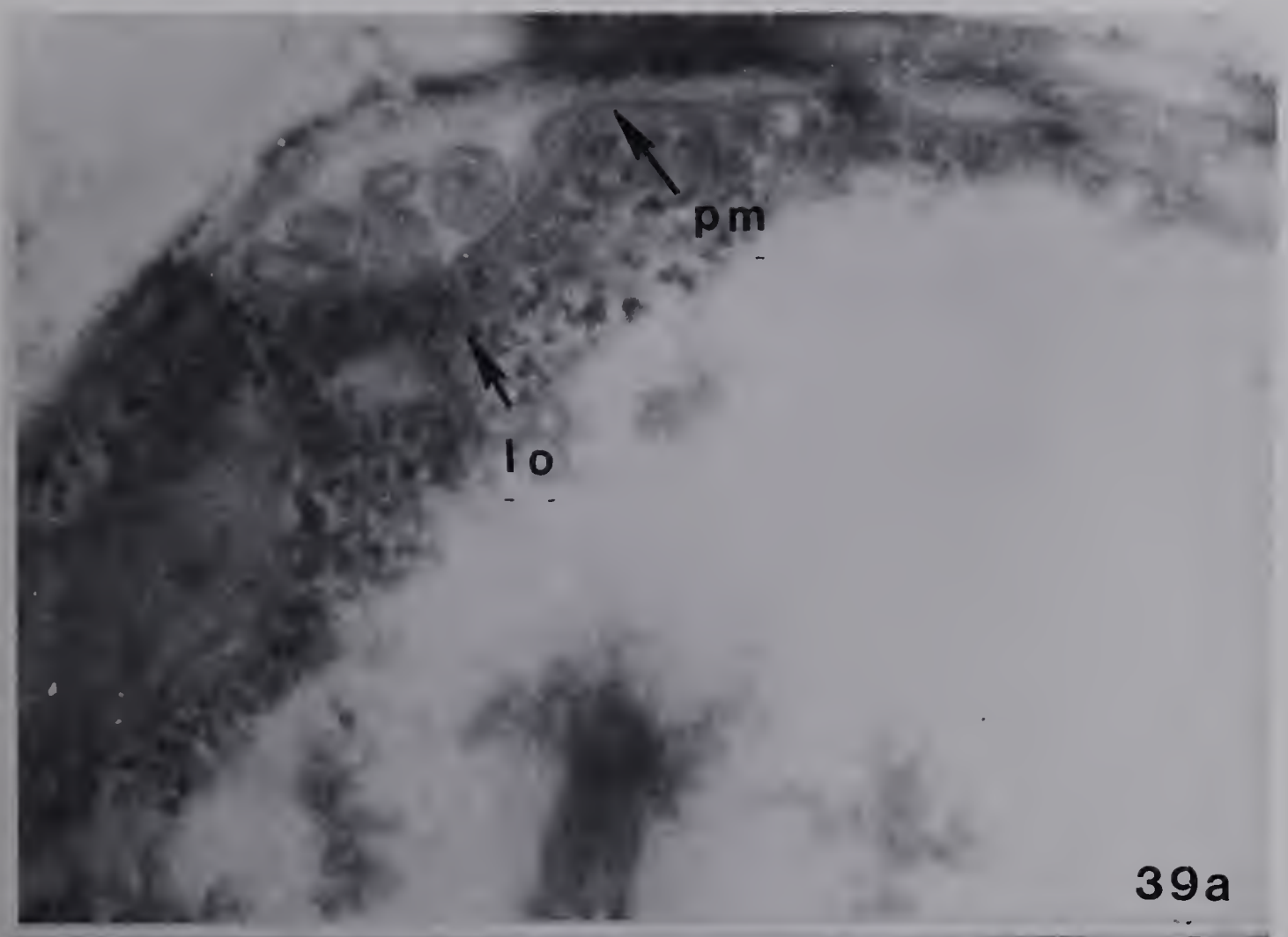

lo
38a


c
pm
b
a
V
38b

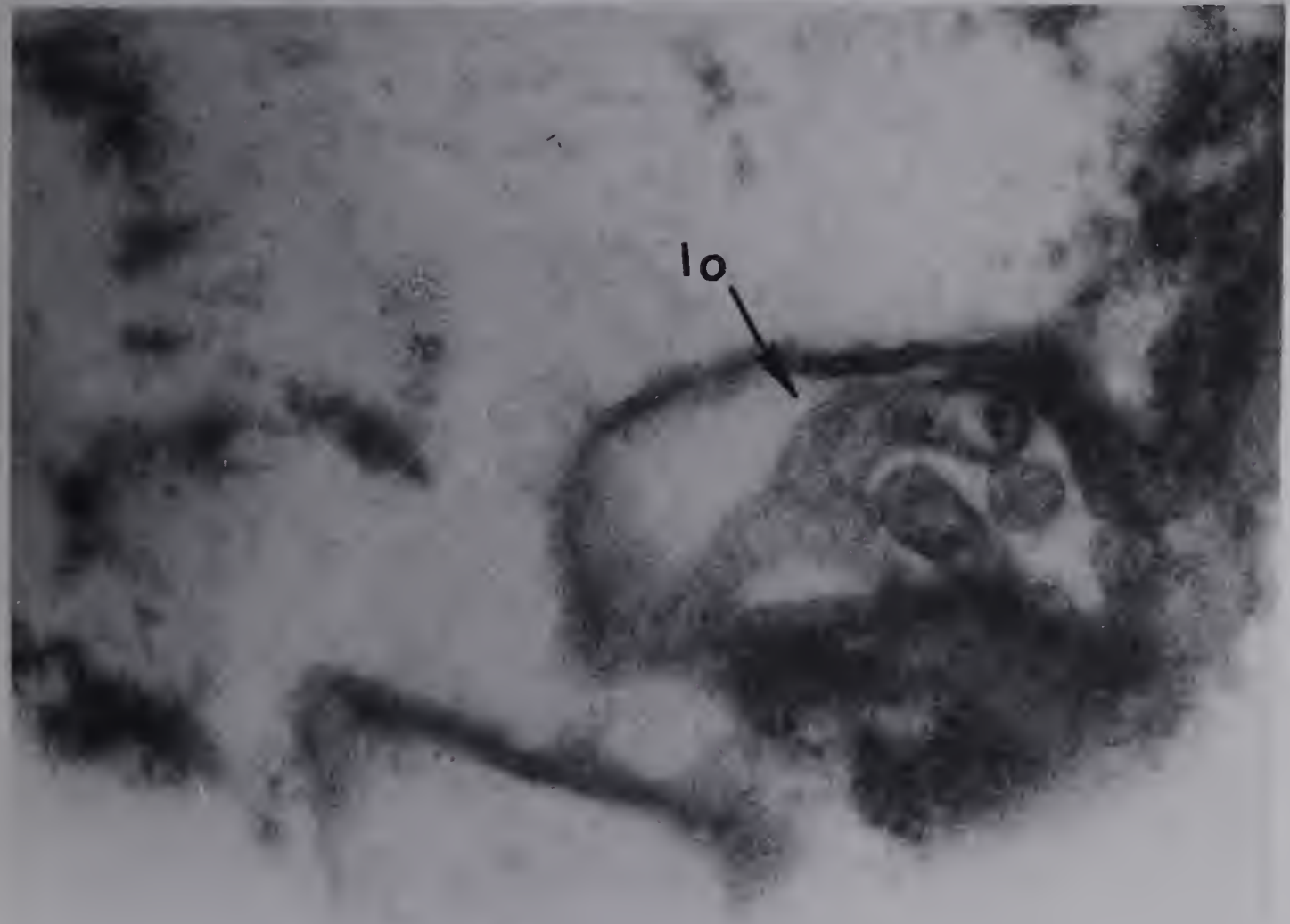
Figures 39a and 39b. Transverse serial sections of a conidium of N. crassa fixed in glutaraldehyde and osmium tetroxide showing lomasomes. (1o) Uranyl acetate and lead citrate-stained.

Figure 39a X 86,000

Figure 39b X 97,000



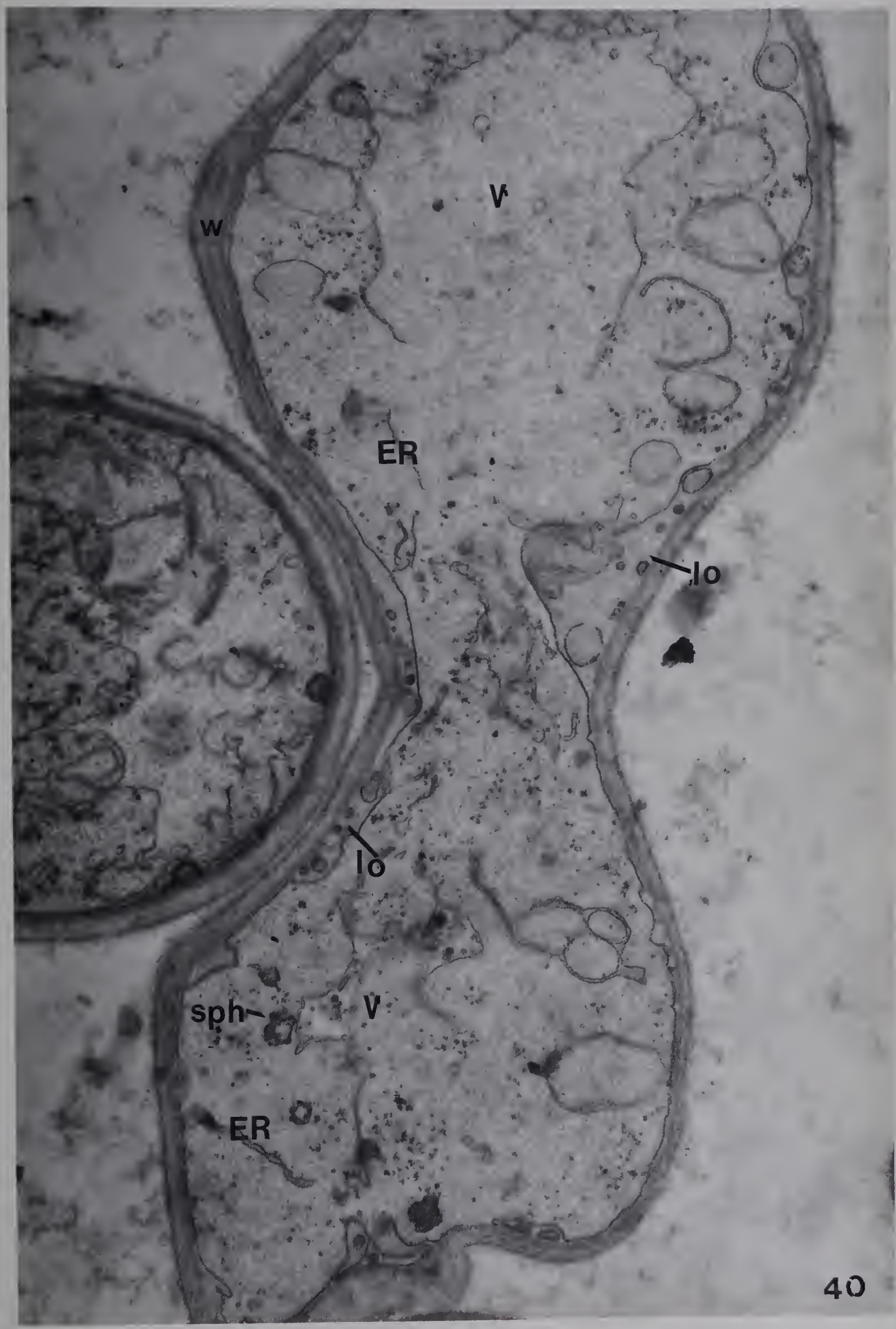
39a



39b

Figue 40. Longitudinal section (a germinated conidium of N. crassa showing the formation of stellate vacuoles (V). Numerous "border bodies" (lomasomes, lo) are also shown. $KMnO_4$ - fixed and lead citrate-stained.

X 34,000



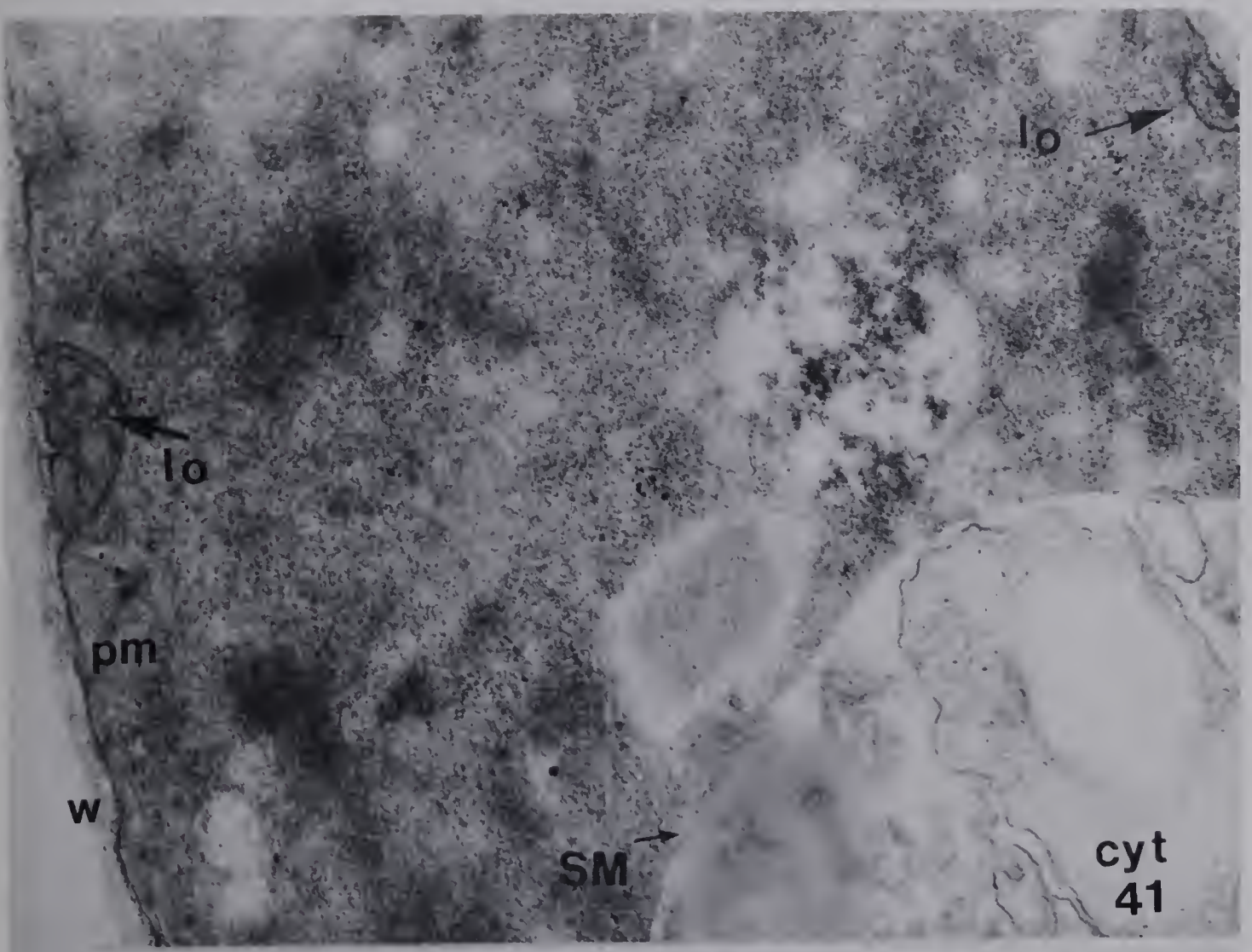
40

Figure 41. An enlarged portion of a germinated conidium showing lomosomes (lo) and a cytolysome (cyt) bounded by a single membrane. Glutaraldehyde and osmium tetroxide fixation followed by staining in uranyl acetate and lead citrate.

X 57,500

Figure 42. Enlarged portion of a germinated conidium showing lomosomes (lo) and a dilation of the endoplasmic reticulum into a vacuole (V). $KMnO_4$-fixed lead citrate-stained.

X 45,000


lo
lo
pm
w
SM
cyt
41

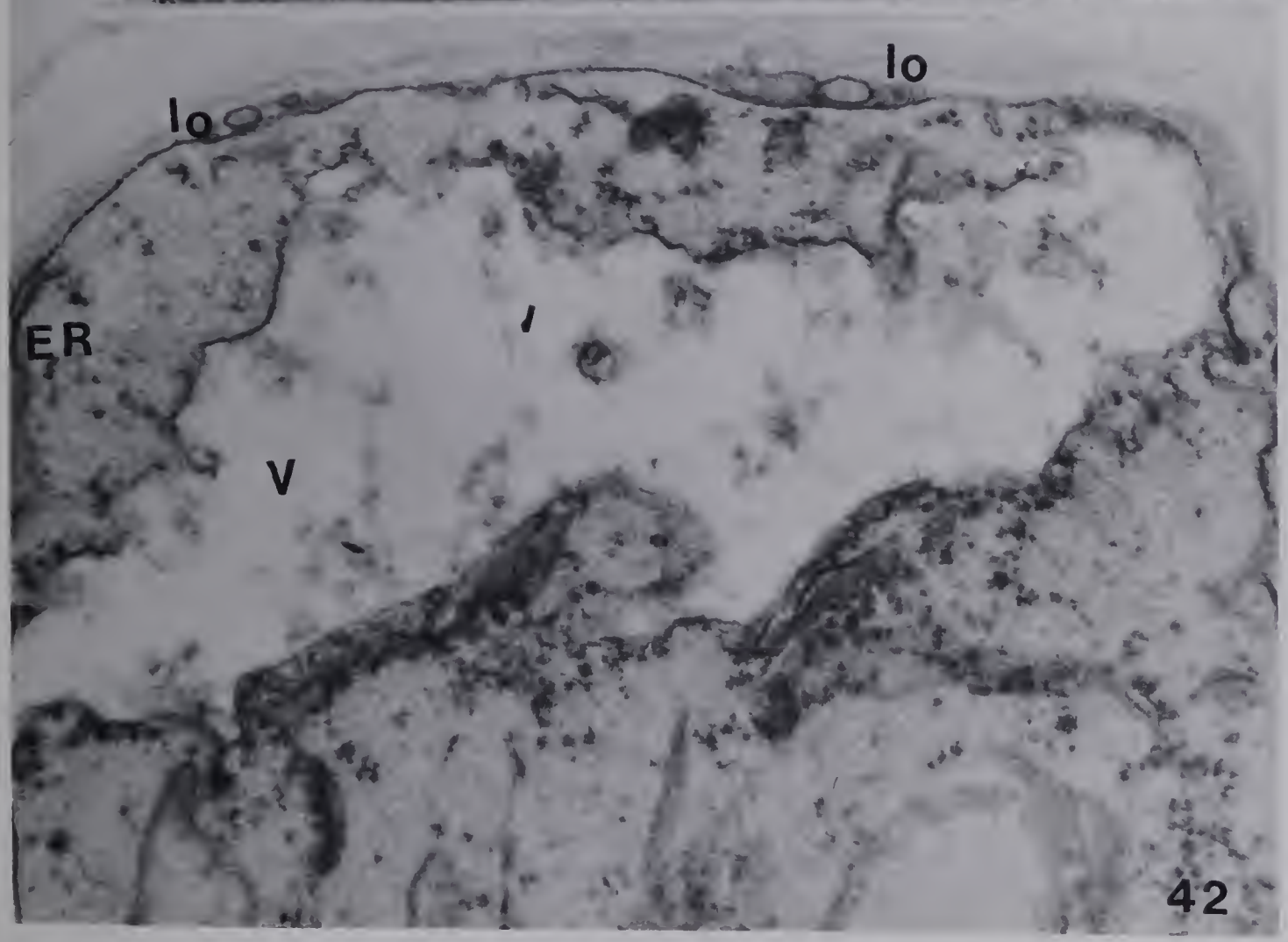

lo
lo
ER
V
42

Figure 43. An enlarged portion of a germinating conidium of N. crassa with the tubular type of mesosome (mes).

Note the osmiophilic bodies (OS) often seen in conjuction with the mesosomes.

Glutaraldehyde and osmium tetroxide fixation.

Stained with uranyl acetate and lead citrate.

X 52,800

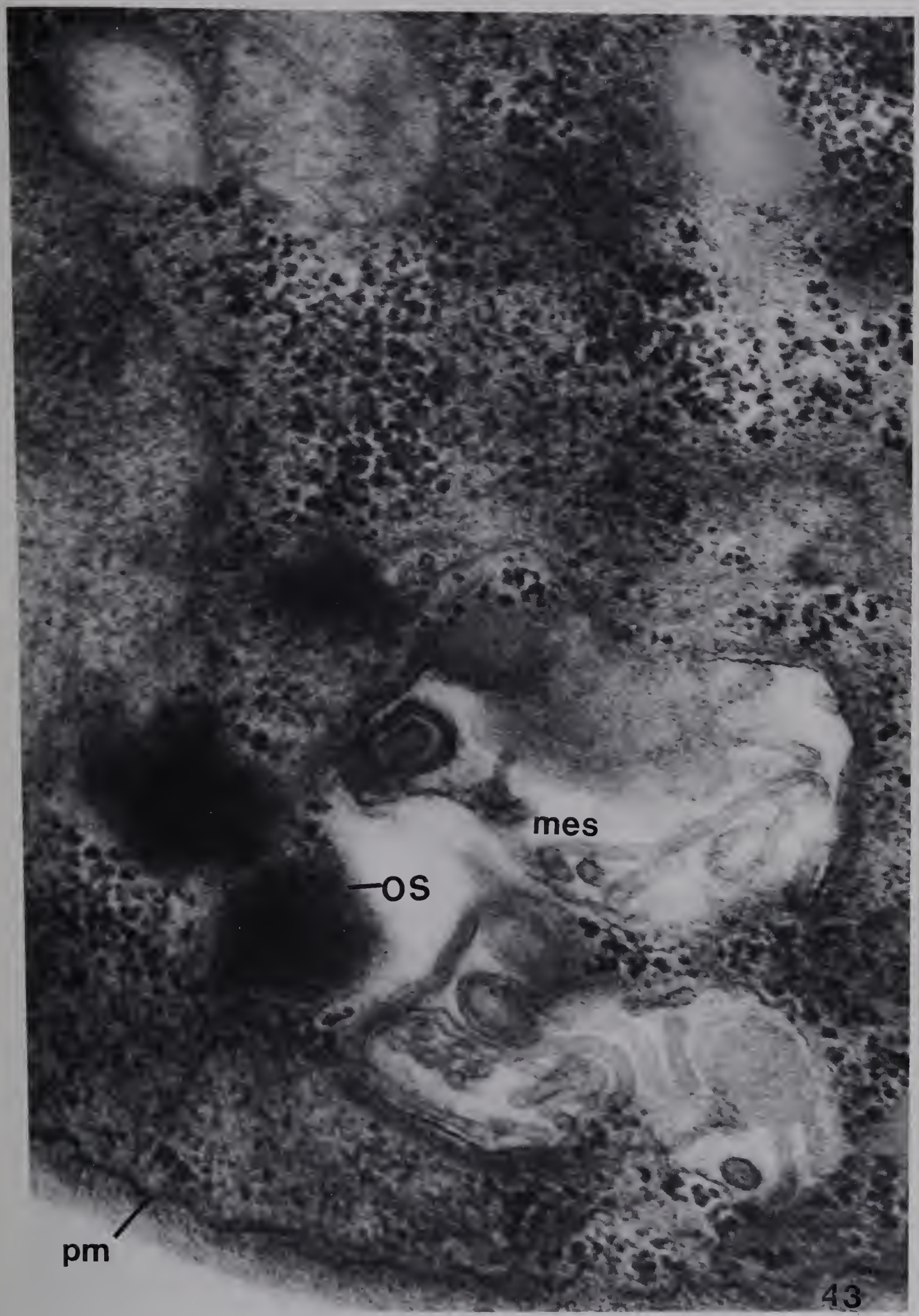

mes
os
pm
43

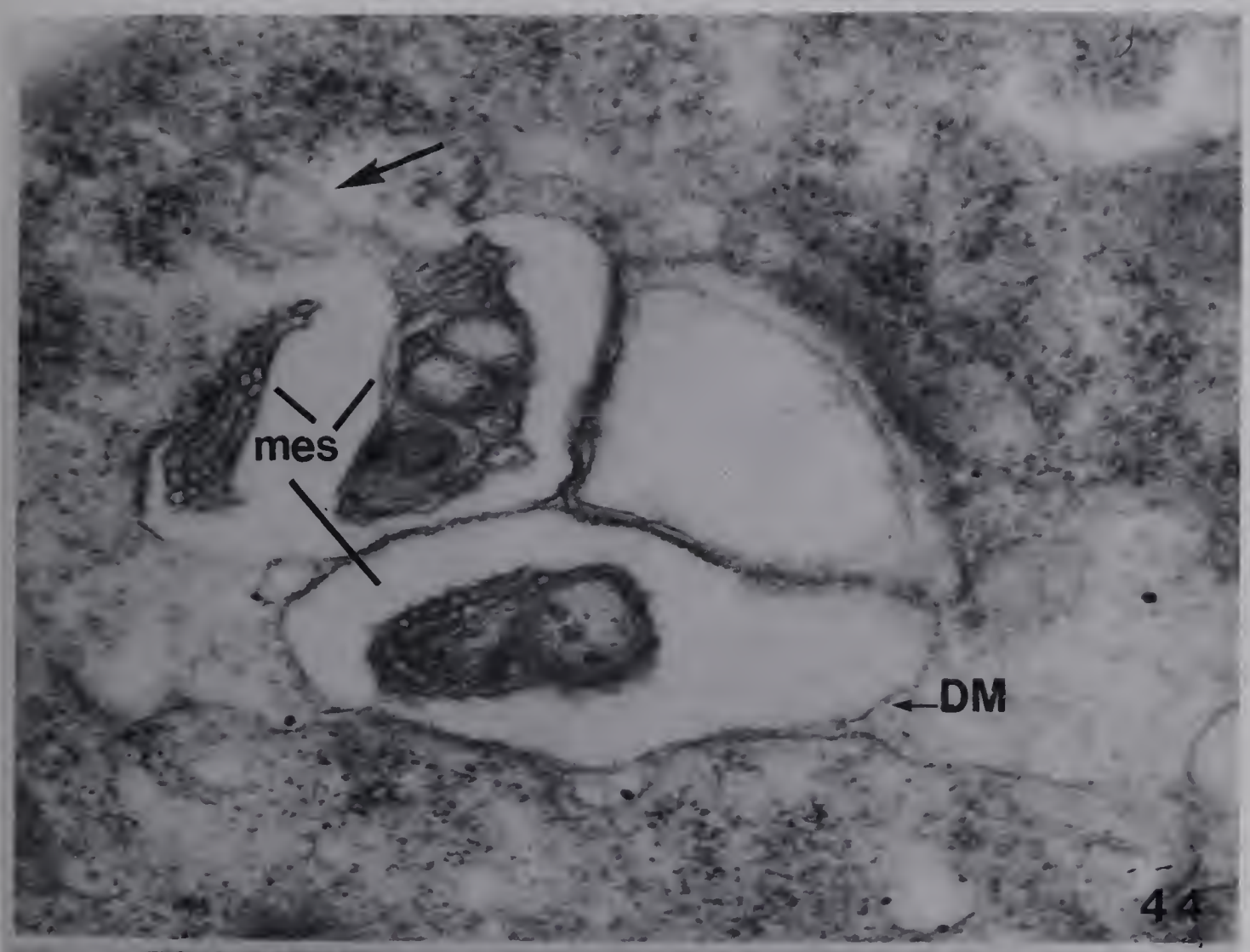
Figure 44. A transverse section of a germinated conidium illustrating a mesosome-like structure (mes). A connection between the mesosome-like structure and the plasma membrane is indicated by the arrow.

X 74,800

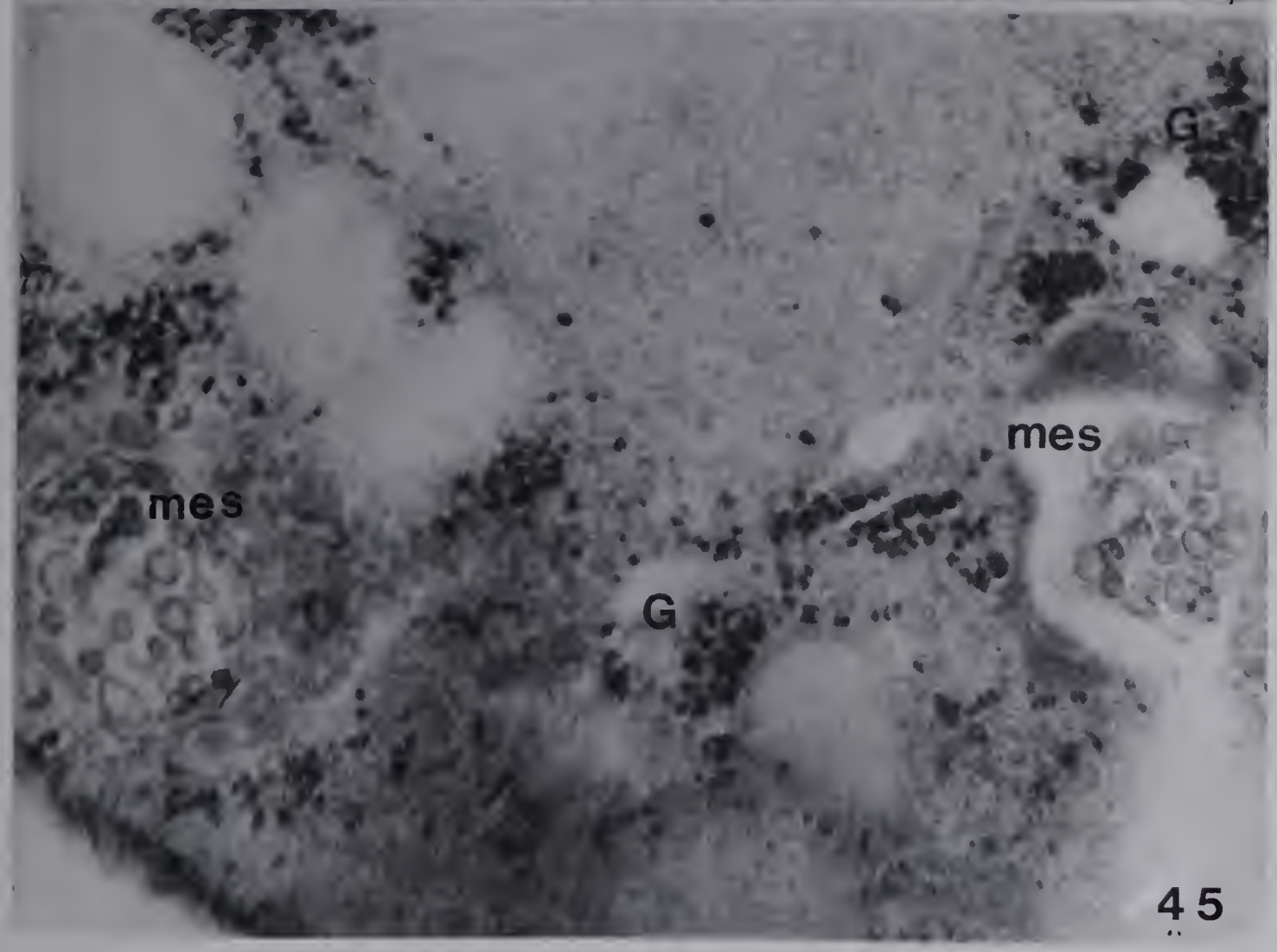
Figure 45. Enlarged portion of longitudinal section of a germinating conidium taken at the neck of the germ tube showing canicular-type of mesosomes. Aggregations of glycogen (G) are prevalent.

X 33,000

Above figures glutaraldehyde and osmium tetroxide-fixed, uranyl acetate and lead citrate-stained.



44



45

Figure 46. Longitudinal section of a germinated conidium illustrating a myelinoid-like mesosome (mes) frequently found near the septal pore (SP)

Glutaradehyde and osmium tetroxide fixation.

Uranyl acetate and lead citrate-stained.

X 35,200

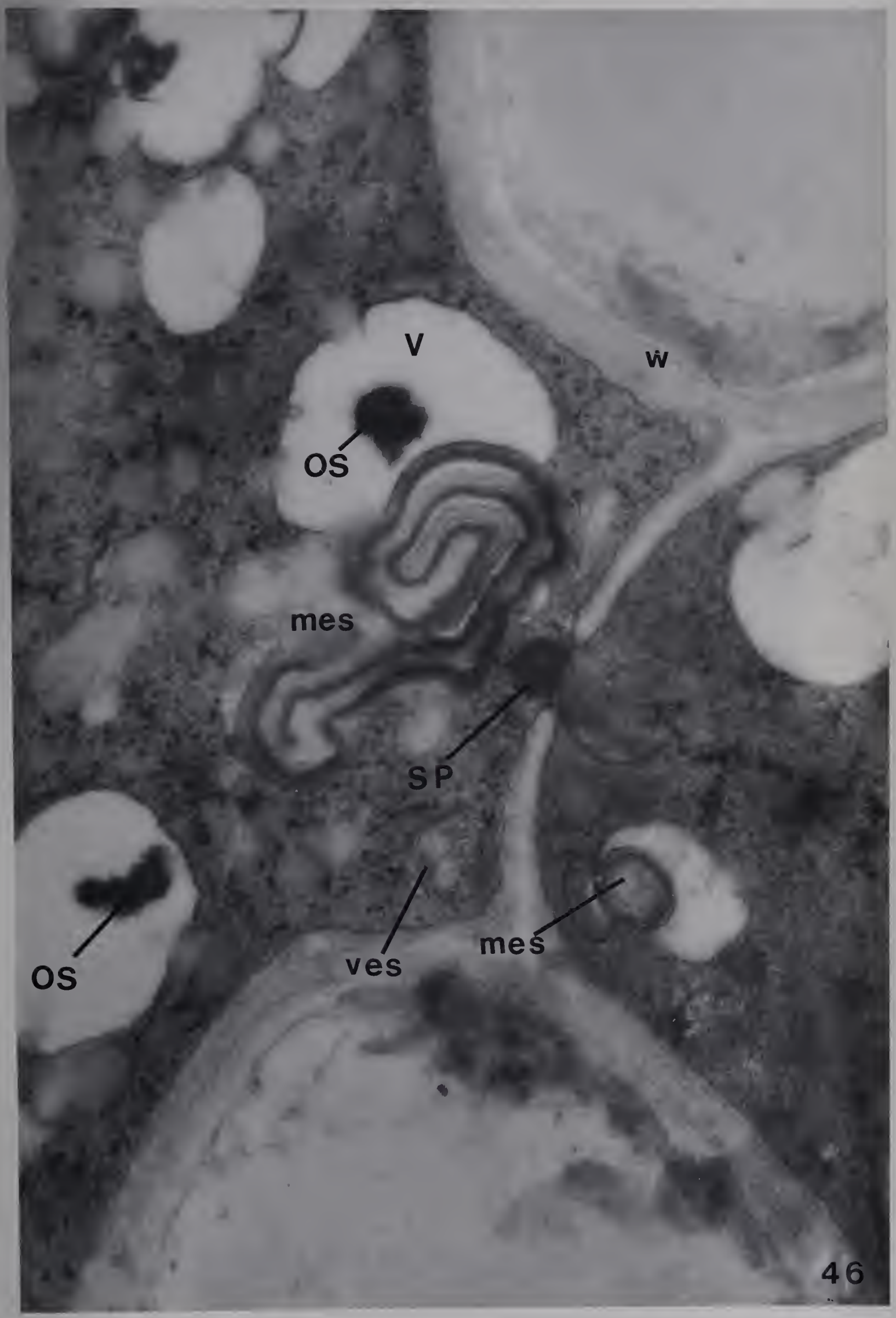

V
W
OS
mes
SP
OS
ves
mes
46

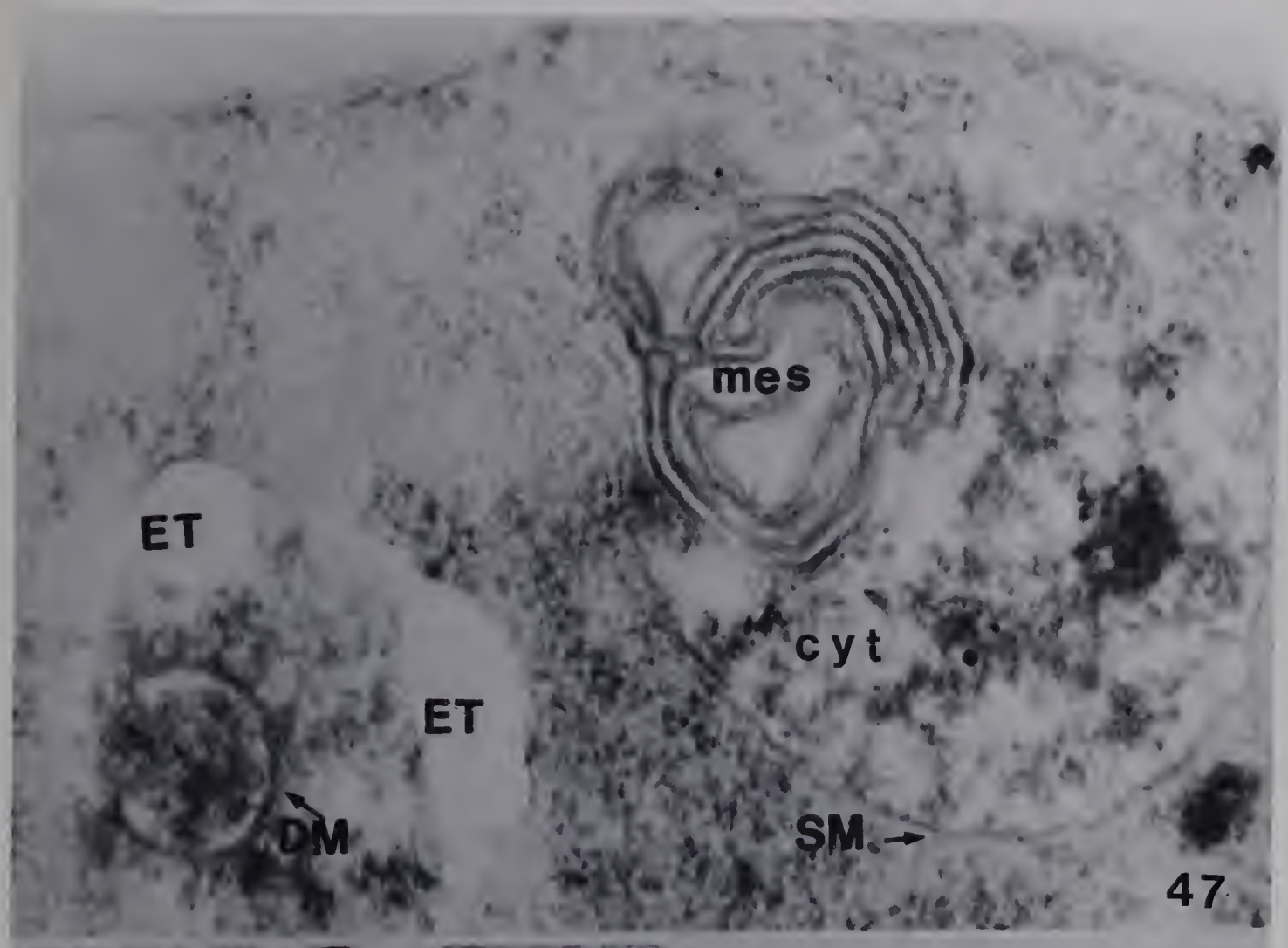
Figure 47. A myelinoid-like mesosome (mes) together with other electron-opaque material in a cytolysome (cyt) bounded by a single membrane (SM). Also shown are several electron-transparent areas (ET)

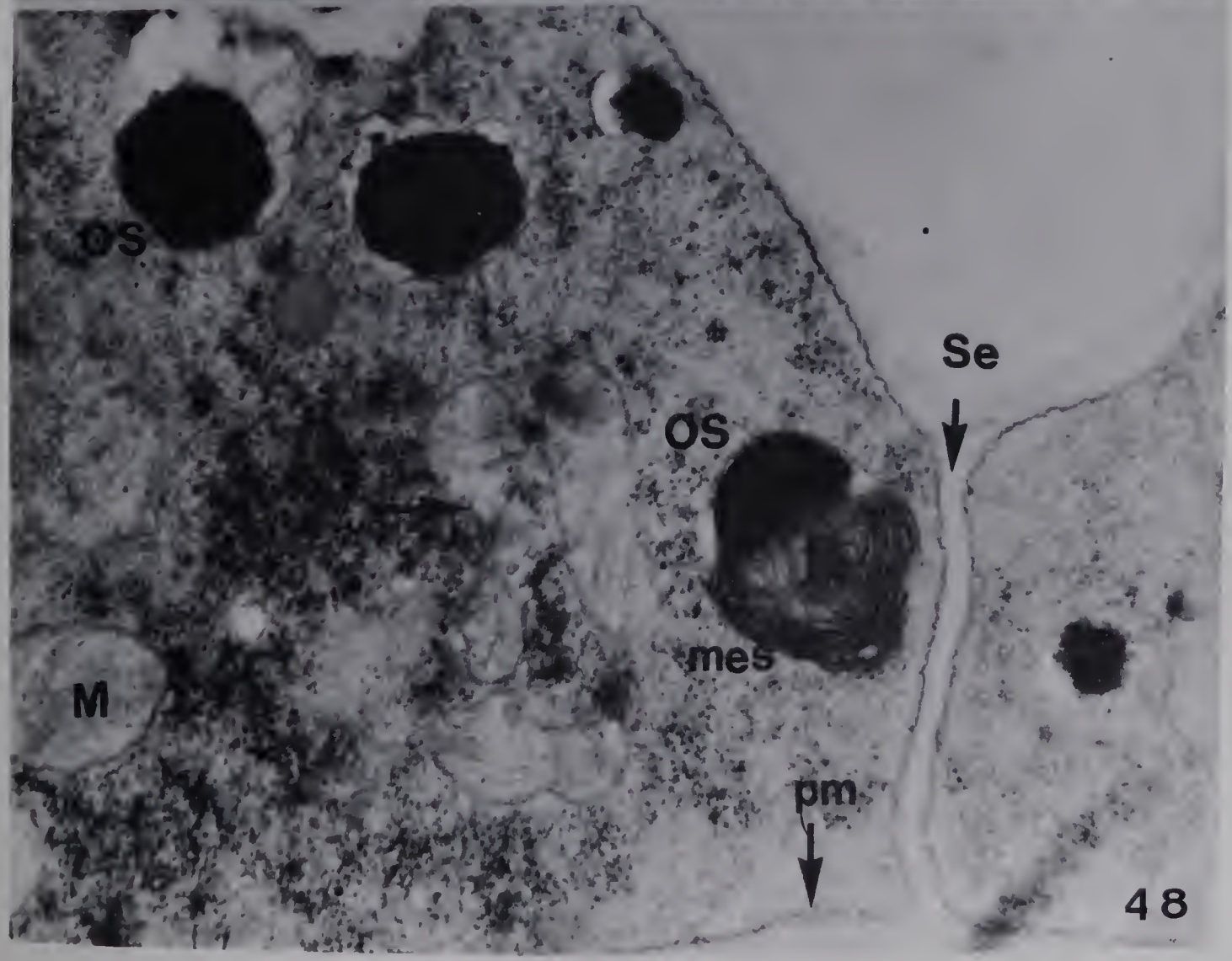
Figure 47 X 86,400

Figure 48. A longitudinal section of a germinating conidium showing the myelinoid-like mesosome frequently found near the septal pore. Arrow indicates a mitochondrion undergoing fission. Also seen are several membrane-bound vacuoles containing osmiophilic bodies (OS).

Figure 48. X 70,400

Figures 47 and 48. Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate and lead citrate-stained.

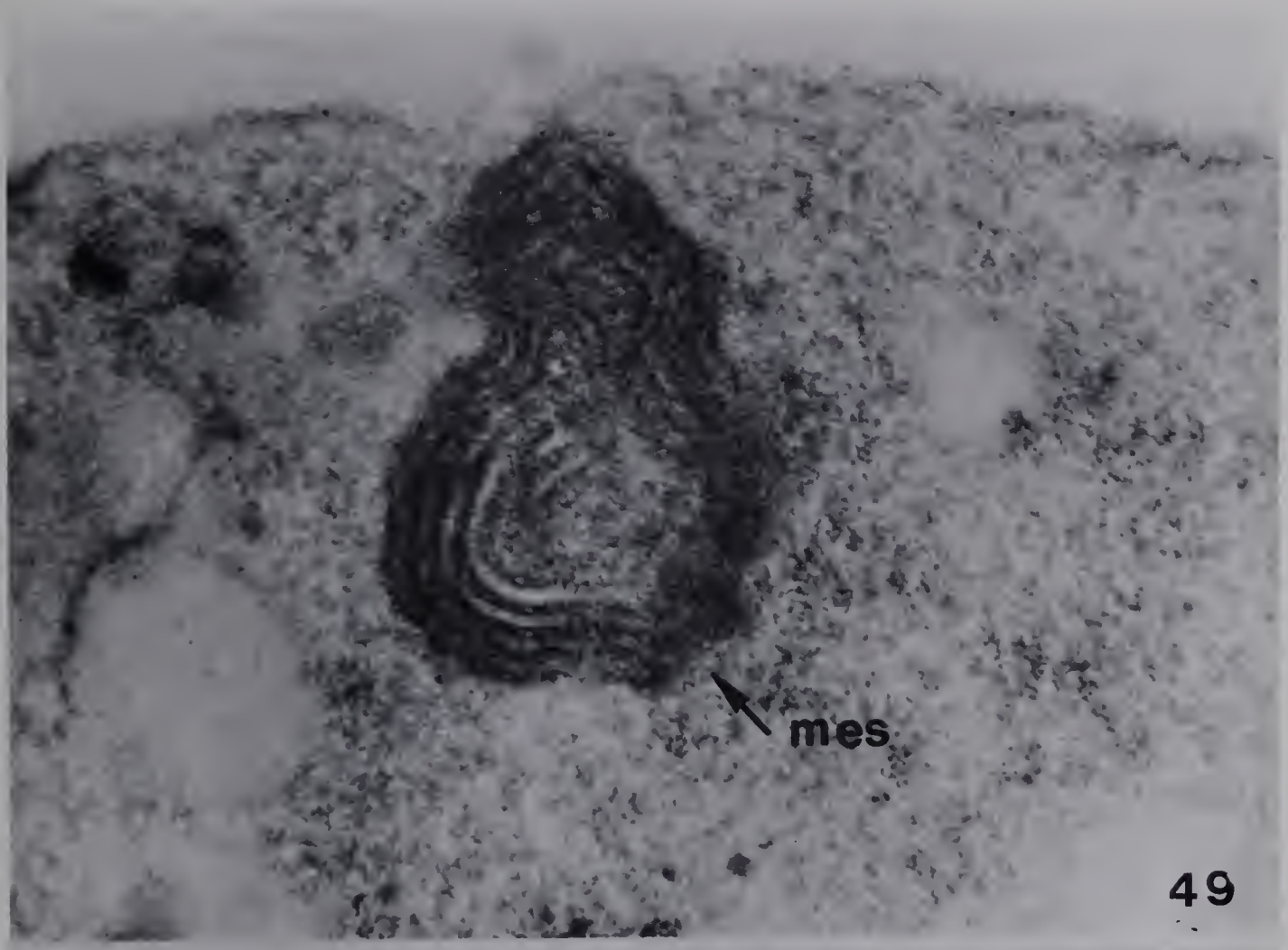

mes
ET
cyt
ET
DM
SM
47


OS
Se
OS
mes
M
pm
48

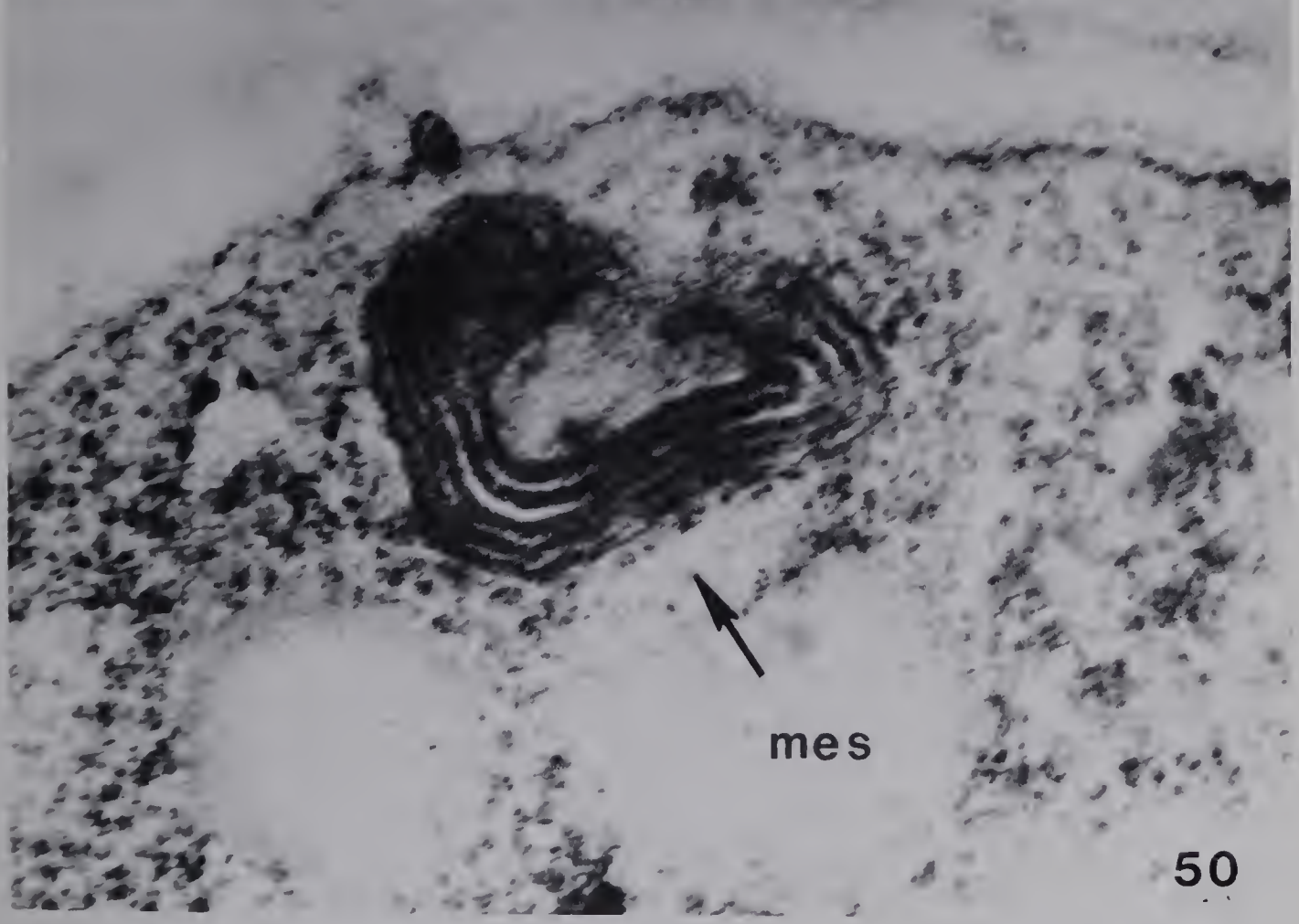
Figures 49 and 50. A micrograph of myelinoid-like mesosomes (mes) in the cytoplasm near the periphery of the conidium. Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate and lead citrate staining.

Figure 49 X 130,000

Figure 50 X 95,000



49

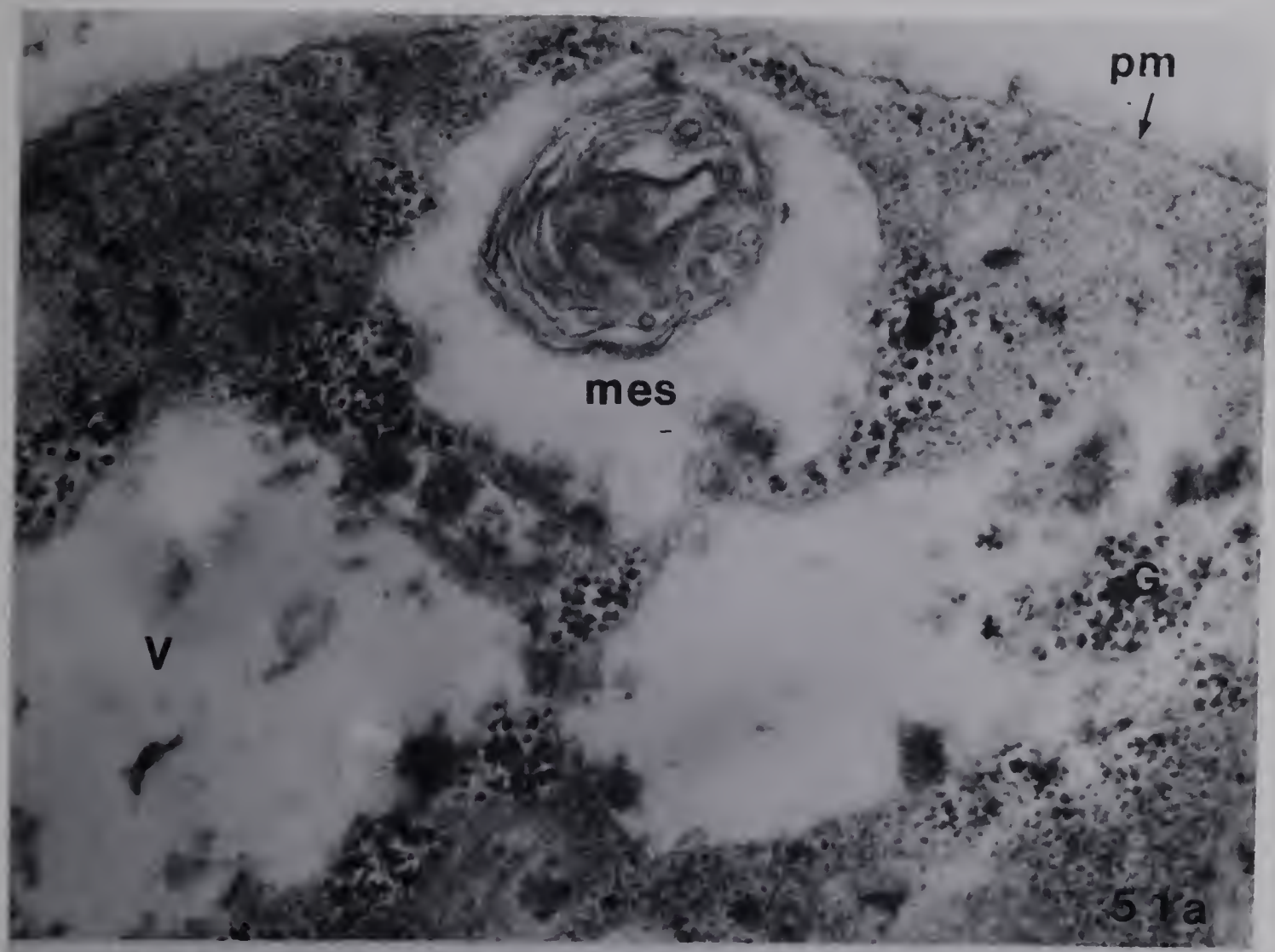


50

Figures 51 a and 51 b. Transverse serial sections of a germinated conidium showing a mesosome (mes).

Glutaraldehyde and osmium tetroxide fixation. Stained with uranyl acetate and lead citrate.

X 60,000

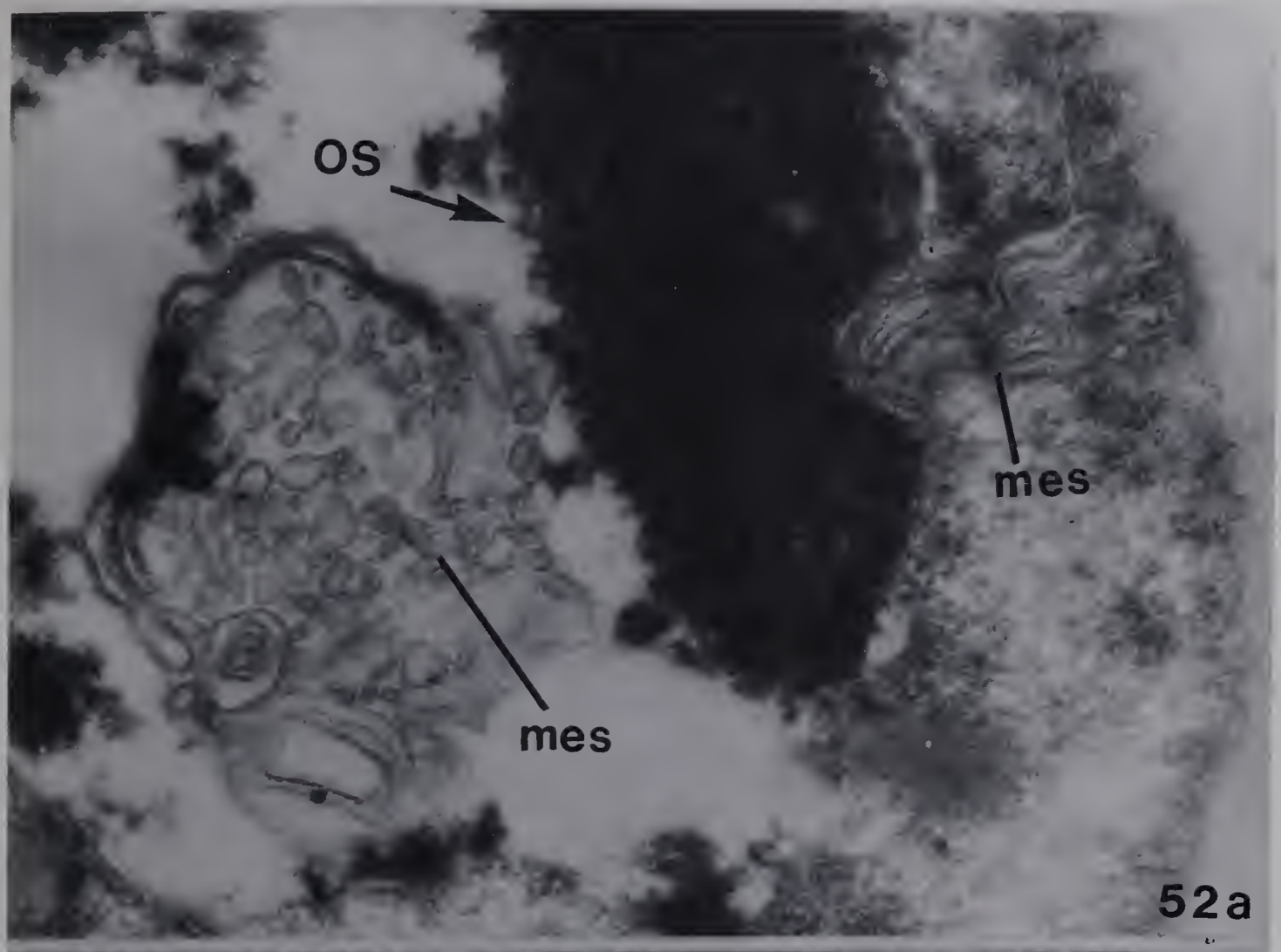

pm
mes
G
V
5 1a


pm
M
G
mes
V
5 1b

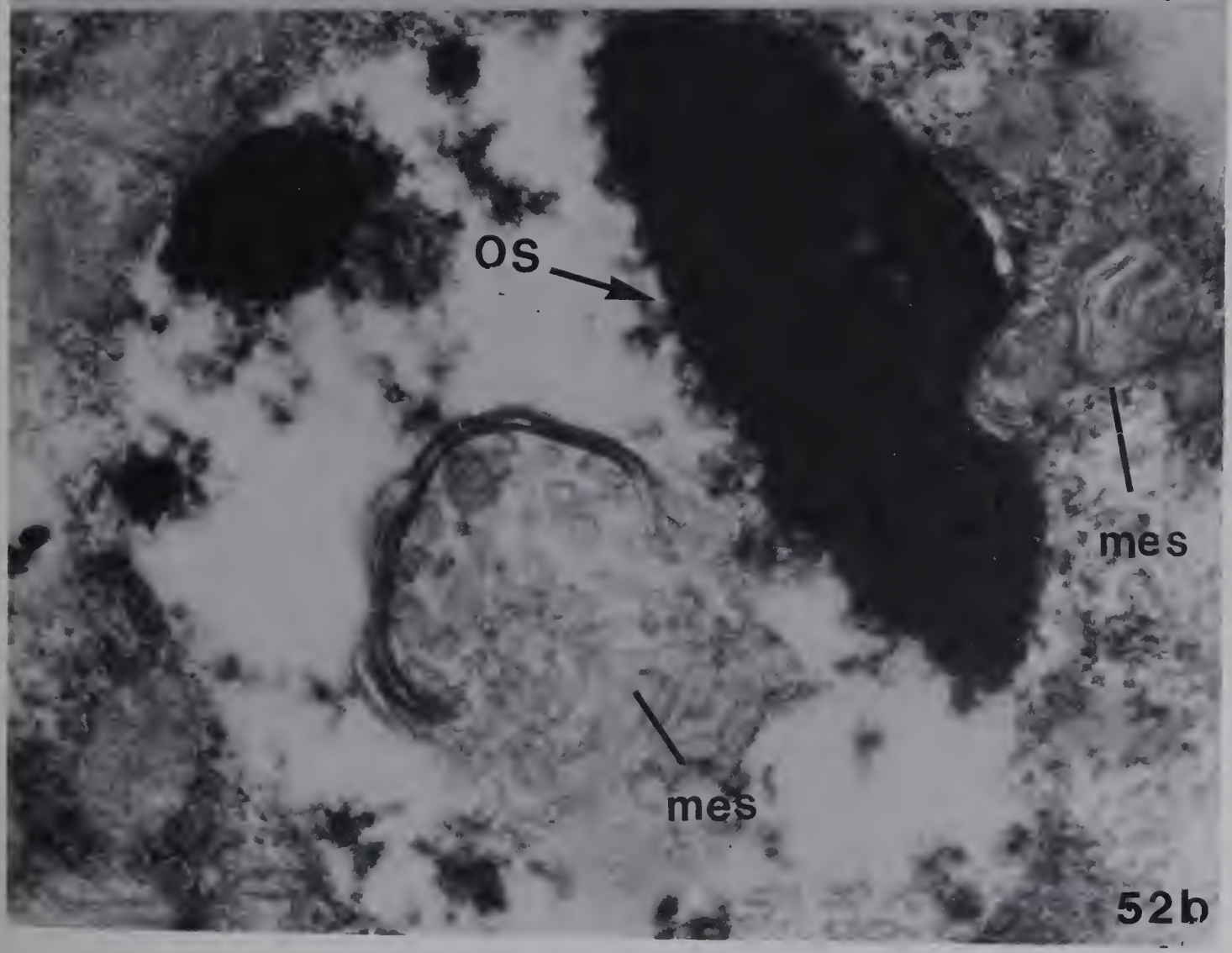
Figures 52 a and 52 b . Transverse serial sections of a germinated conidium illustrating myelinoid and tubular types of mesosomes (mes). Also seen in the glutaraldehyde and osmium tetroxide-fixed and uranyl acetate and lead citrate-stained sections, are large osmiophilic bodies (OS).

Figure 52a X 85,500

Figure 52b X 78,300



52a



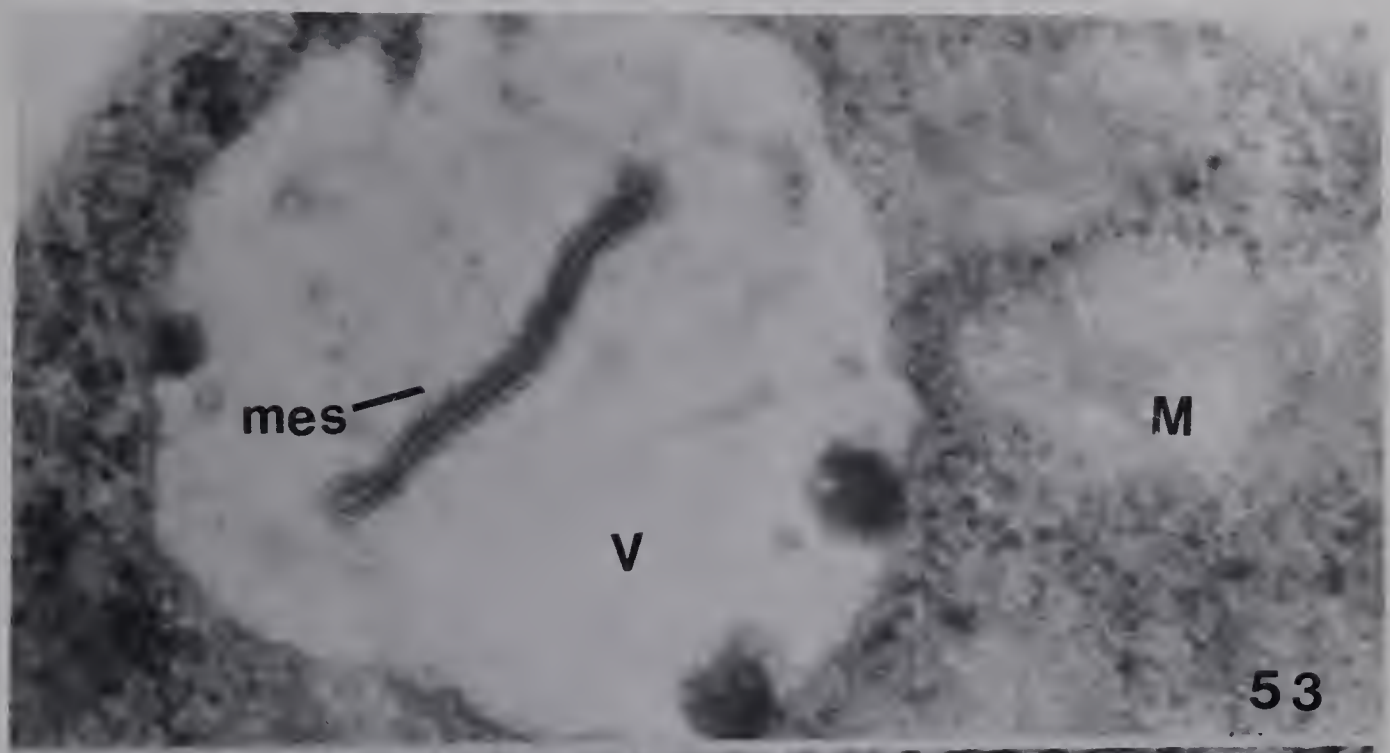
52b

Figures 53, 54 and 55, are micrographs illustrating myelinoid-like mesosomes (mes) frequently found in germinating conidia. Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate and lead citrate-stained.

Figure 53 X 72,000

Figure 54 X 87,600

Figure 55 X 58,000


mes
M
V
53


mes
V
54

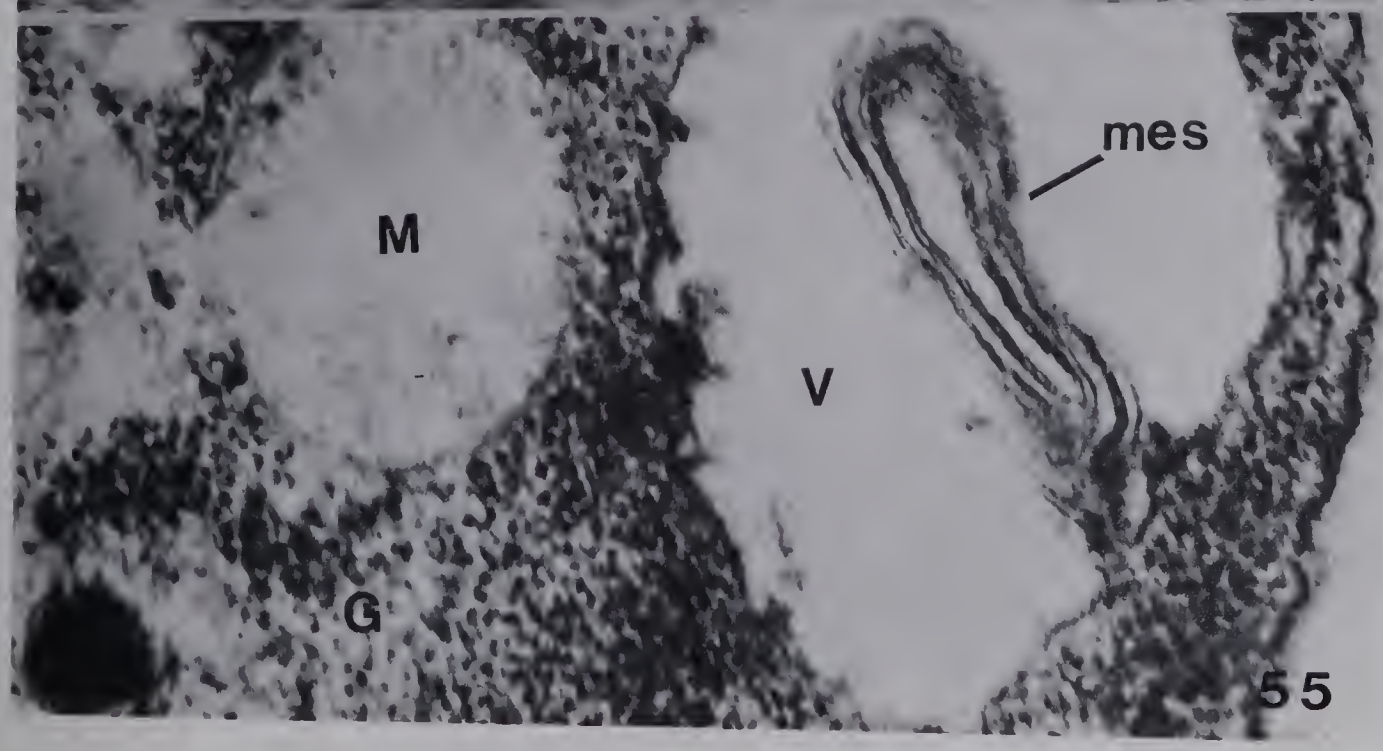

mes
M
V
G
55

Figure 56. A highly magnified portion of a germinating conidium of N. crassa depicting two myelenoid-type of mesosomes (mes). The mesosomes in the upper portion of the micrograph is interpreted as showing a cross sectional view while the one in the lower portion of the micrograph shows a longitudinal view. Glutaraldehyde and osmium tetroxide fixation.

X 72,000

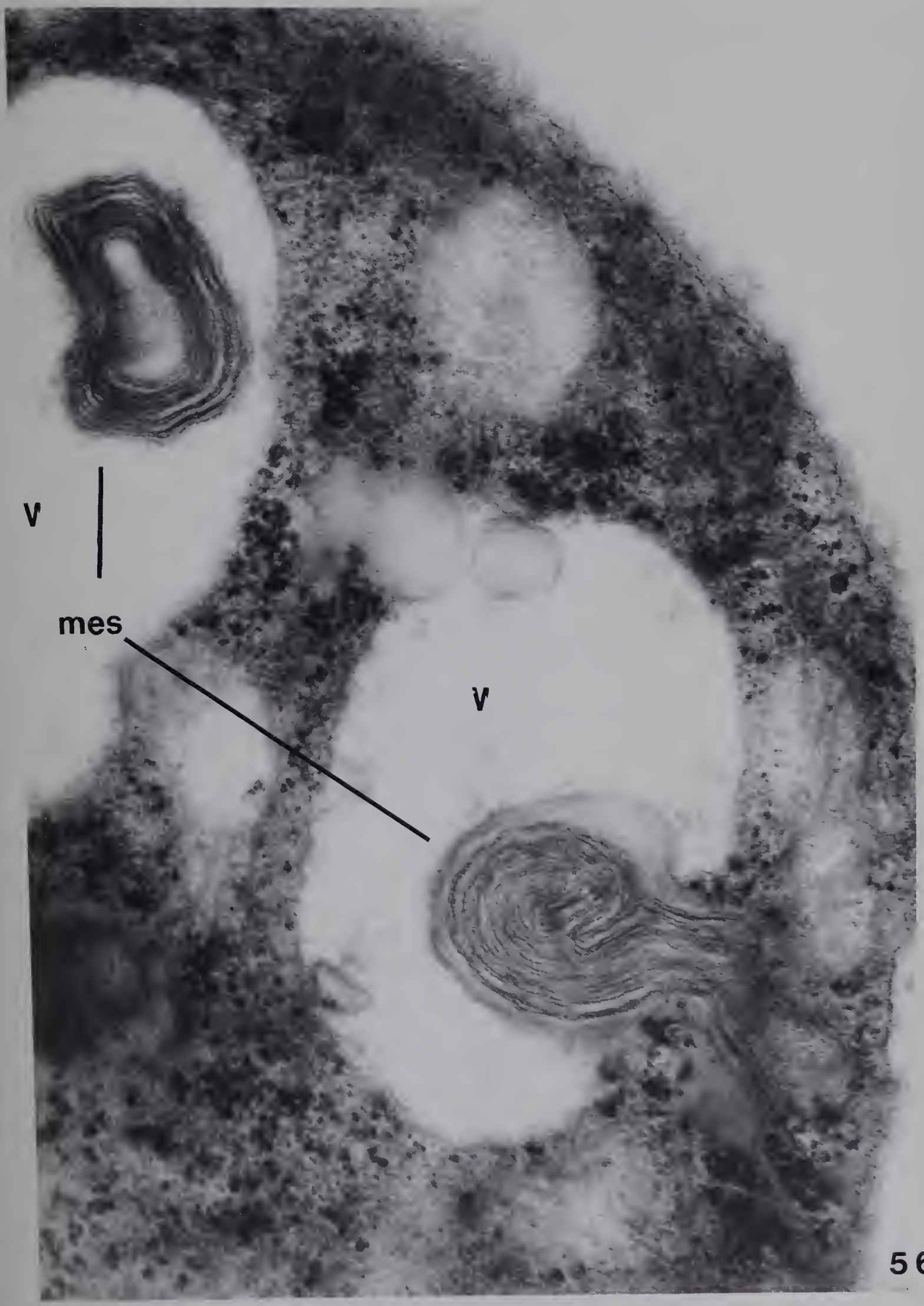
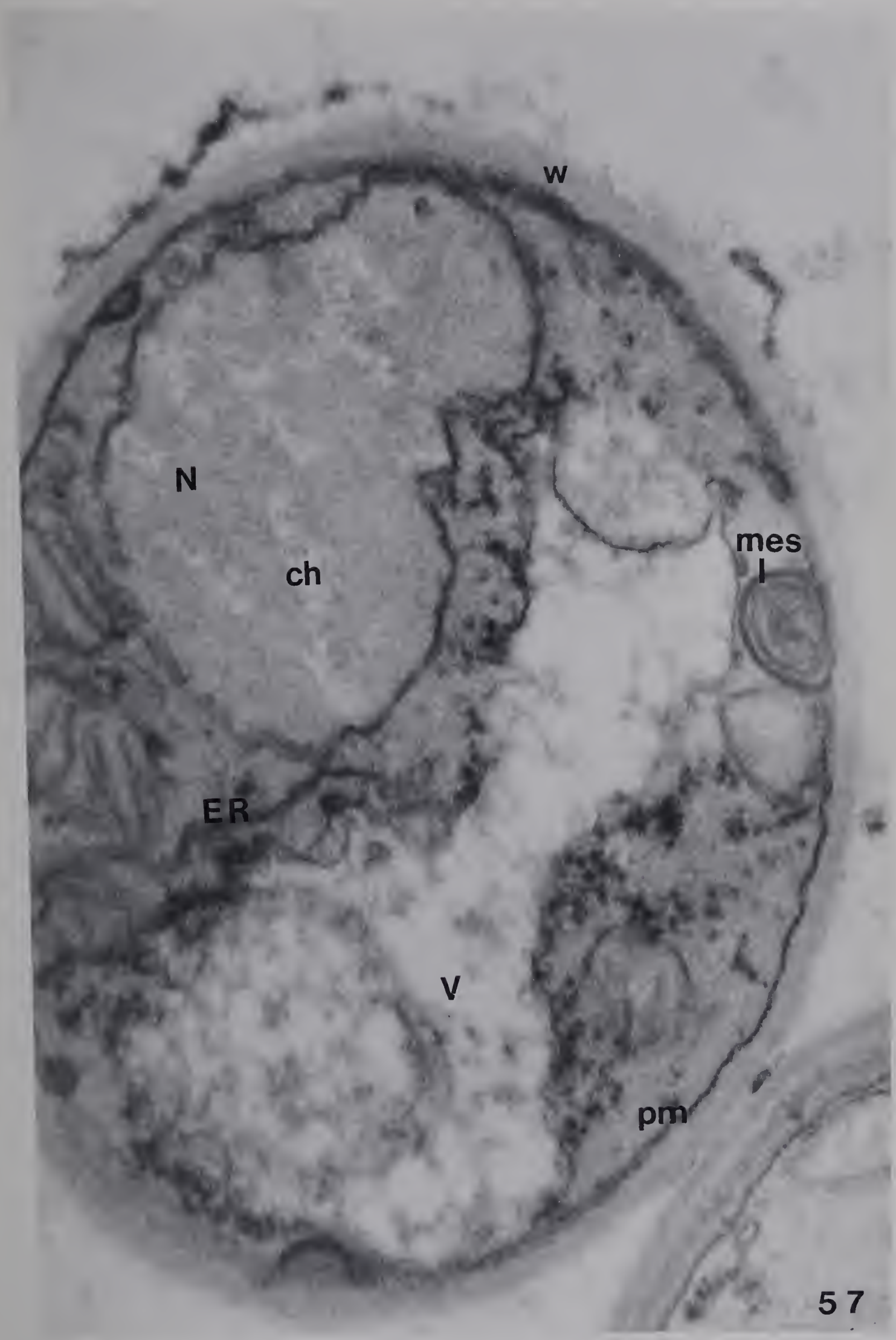

v
mes
v

Figure 57. Transverse section of a germinating conidium fixed in $KMnO_4$ and stained in lead citrate illustrating a mesosome (mes) and a stellate type of vacuole (V).

X 70,000

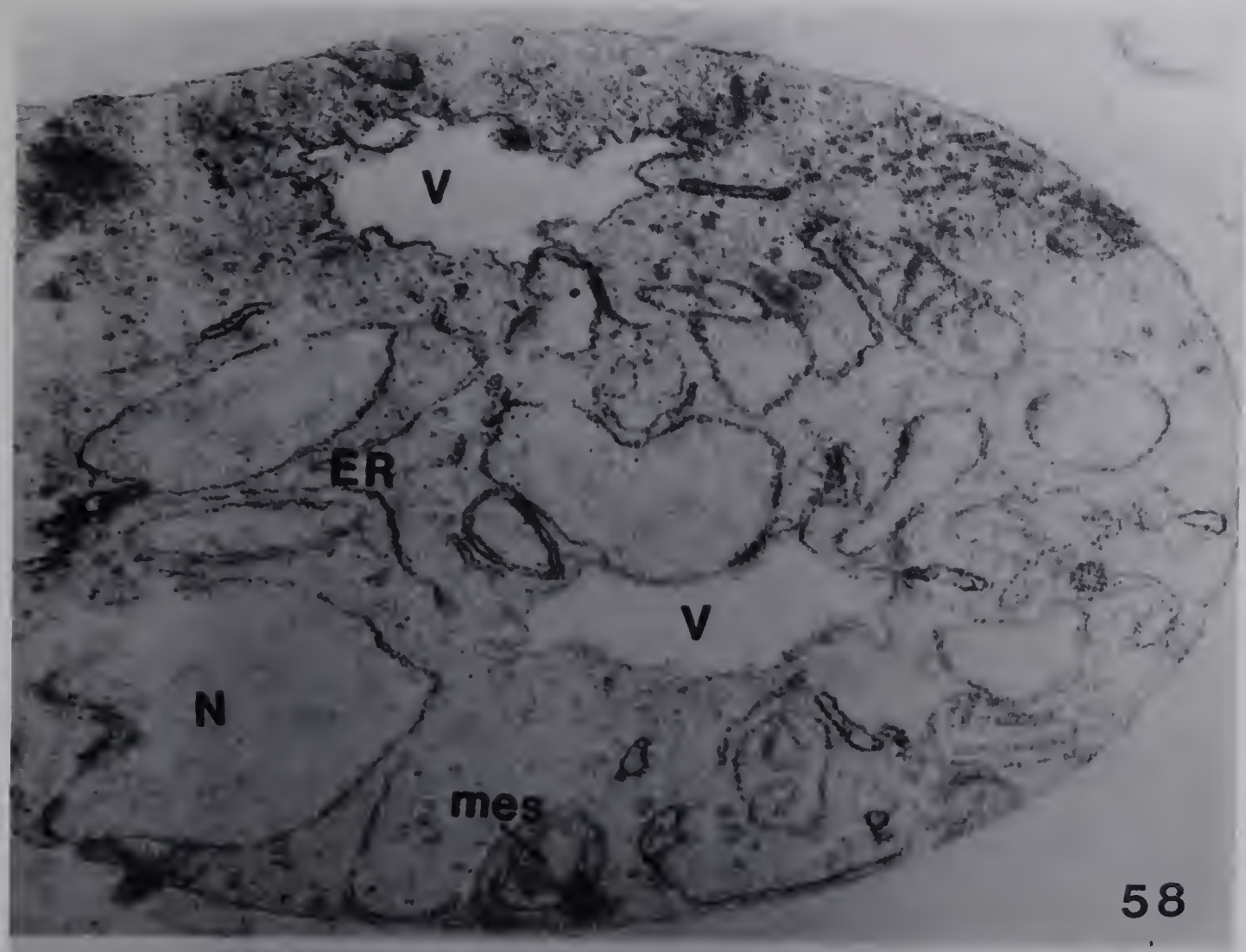


57

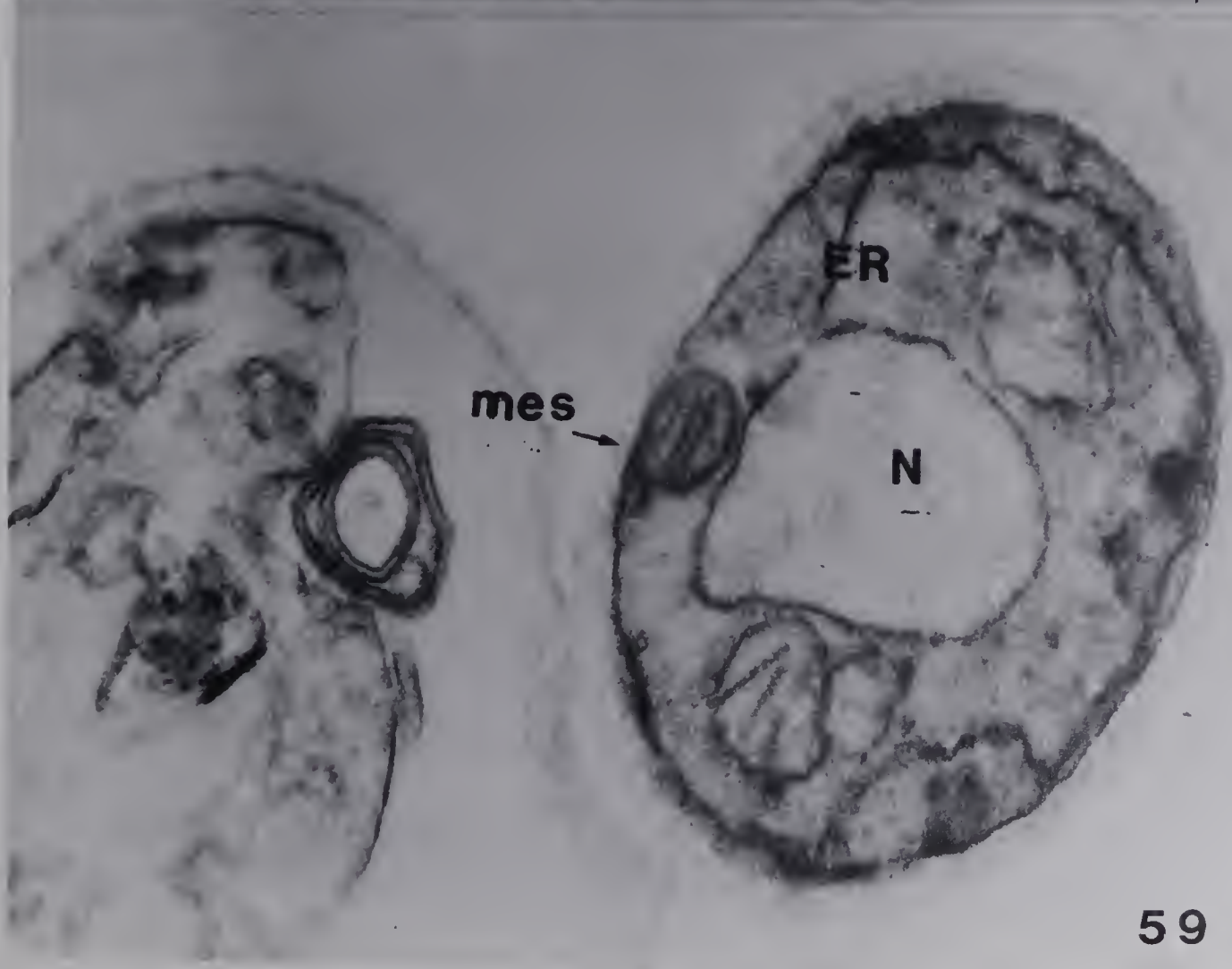
Figures 58 and 59. Longitudinal and transverse sections respectively, of germinated conidia fixed in $KMnO_4$ and stained in lead citrate containing mesosome-like structures (mes)

Figure 58 X 76,000

Figure 59 X 49,600

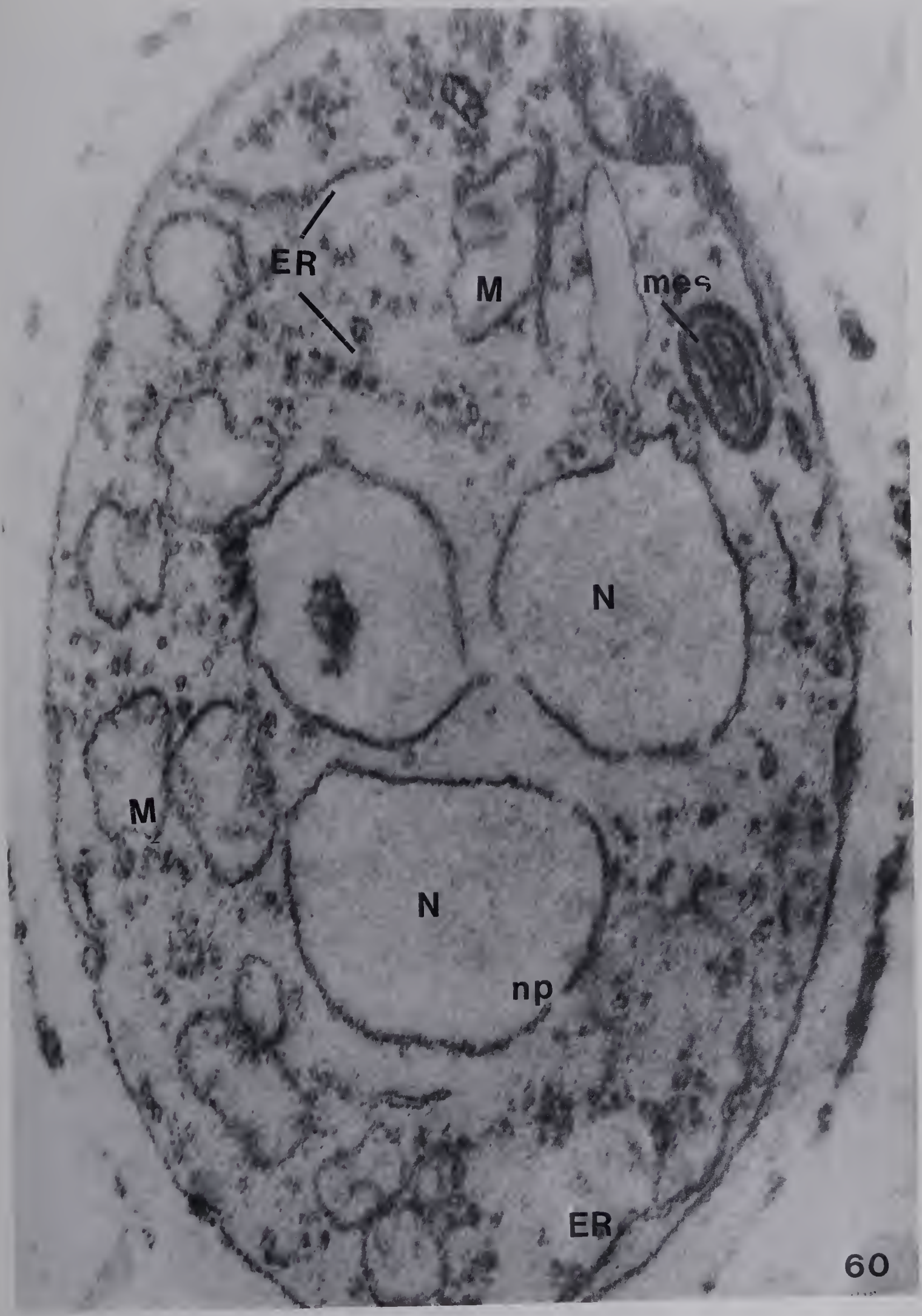


58



59

Figure 60. A $KMnO_4$ - fixed conidium of N. crassa showing a mesosome (mes) and a number of nuclear pores (np).

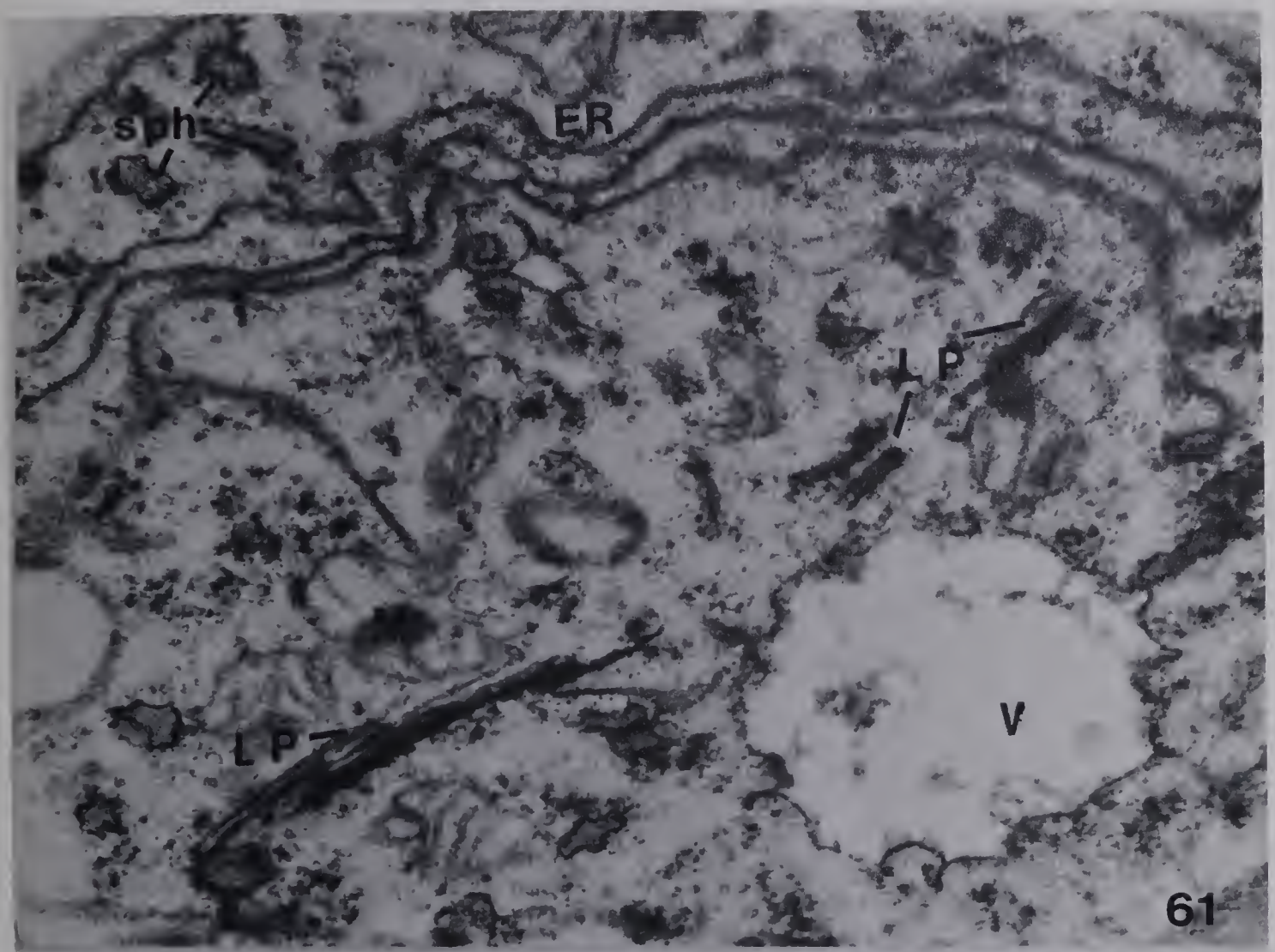


60

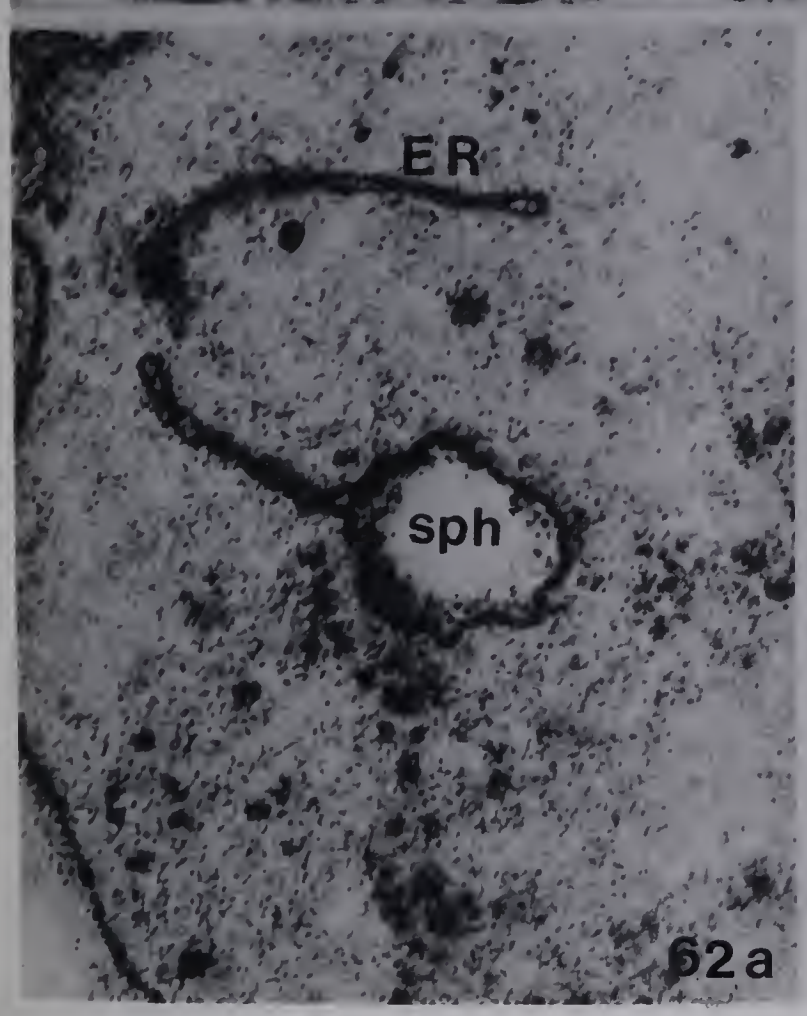
Figure 61. A transverse section of a germinated conidium showing lipoprotein sheets (LP), spherosomes (sph) and endoplasmic reticulum (ER).
X 58,500

Figure 62 a and 62 b . An enlarged portion of transverse sections of conidia showing spherosomes (sph) as a terminal dilation of ER.
Figure X 87,000
Figure X 96,800

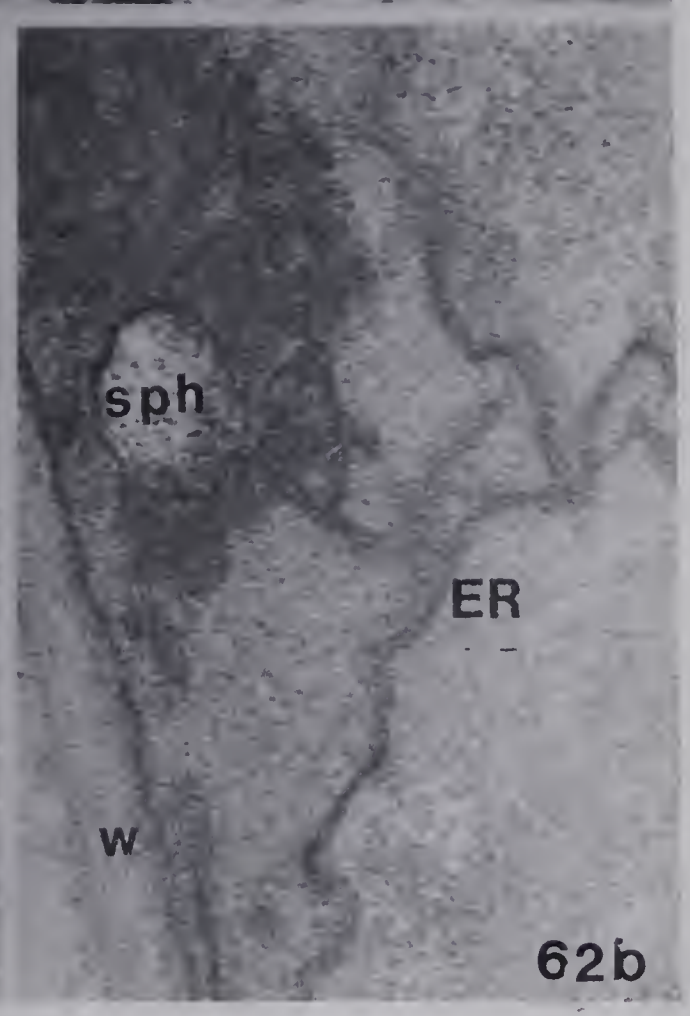
Figures 61 and 62 a and b. $KMnO_4$ - fixed and lead citrate-stained.



61



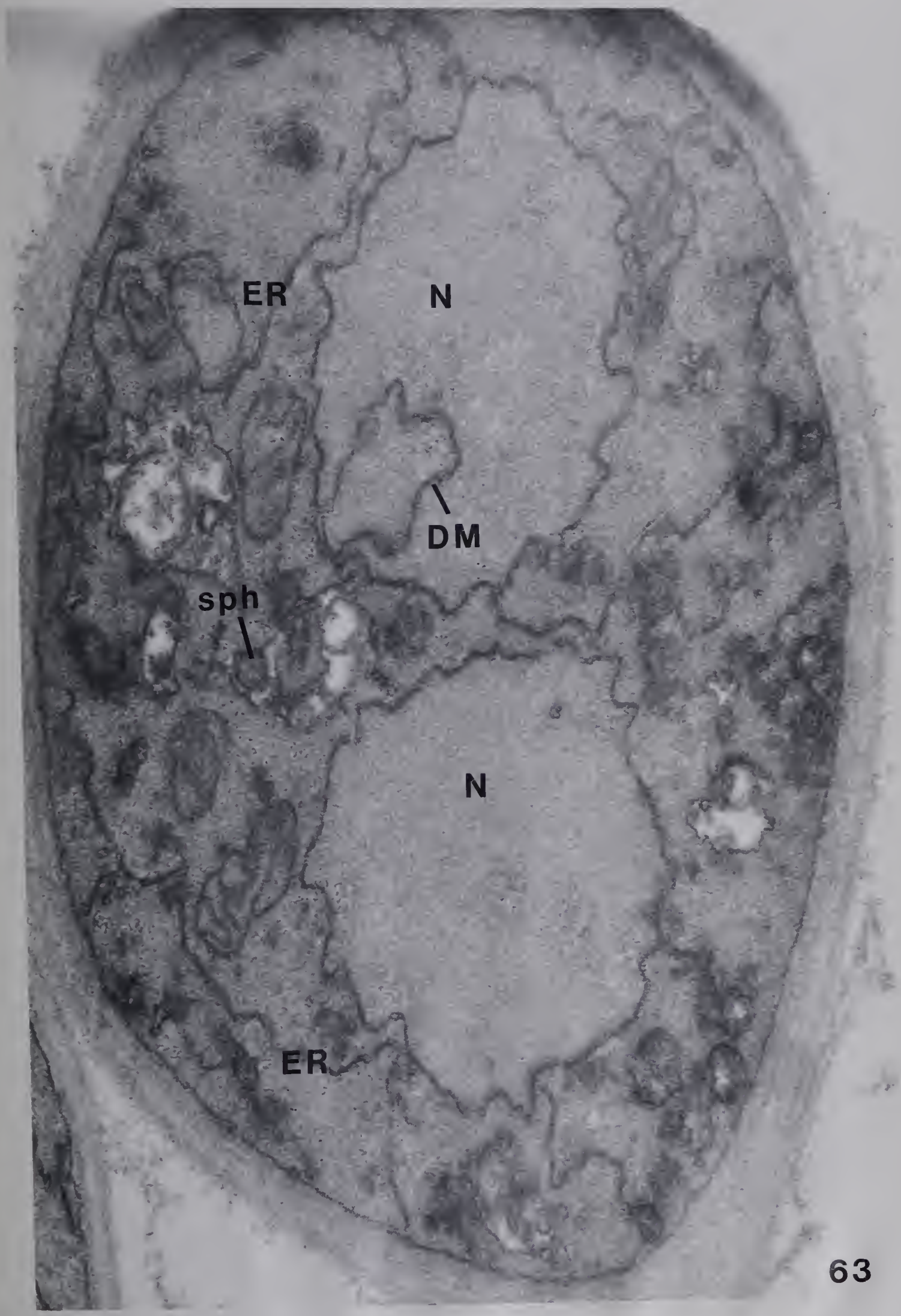
62a



62b

Figure 63. A $KMnO_4$ - fixed and lead citrate-stained conidium just entering the germination phase. The double-membraned structure in the upper nucleus may be an invagination of the nucleus. A number of spherosomes (sph) can be seen in the cytoplasmic matrix. The arrow in the lower portion of the nucleus points to the continuity of the ER with the nucleus.

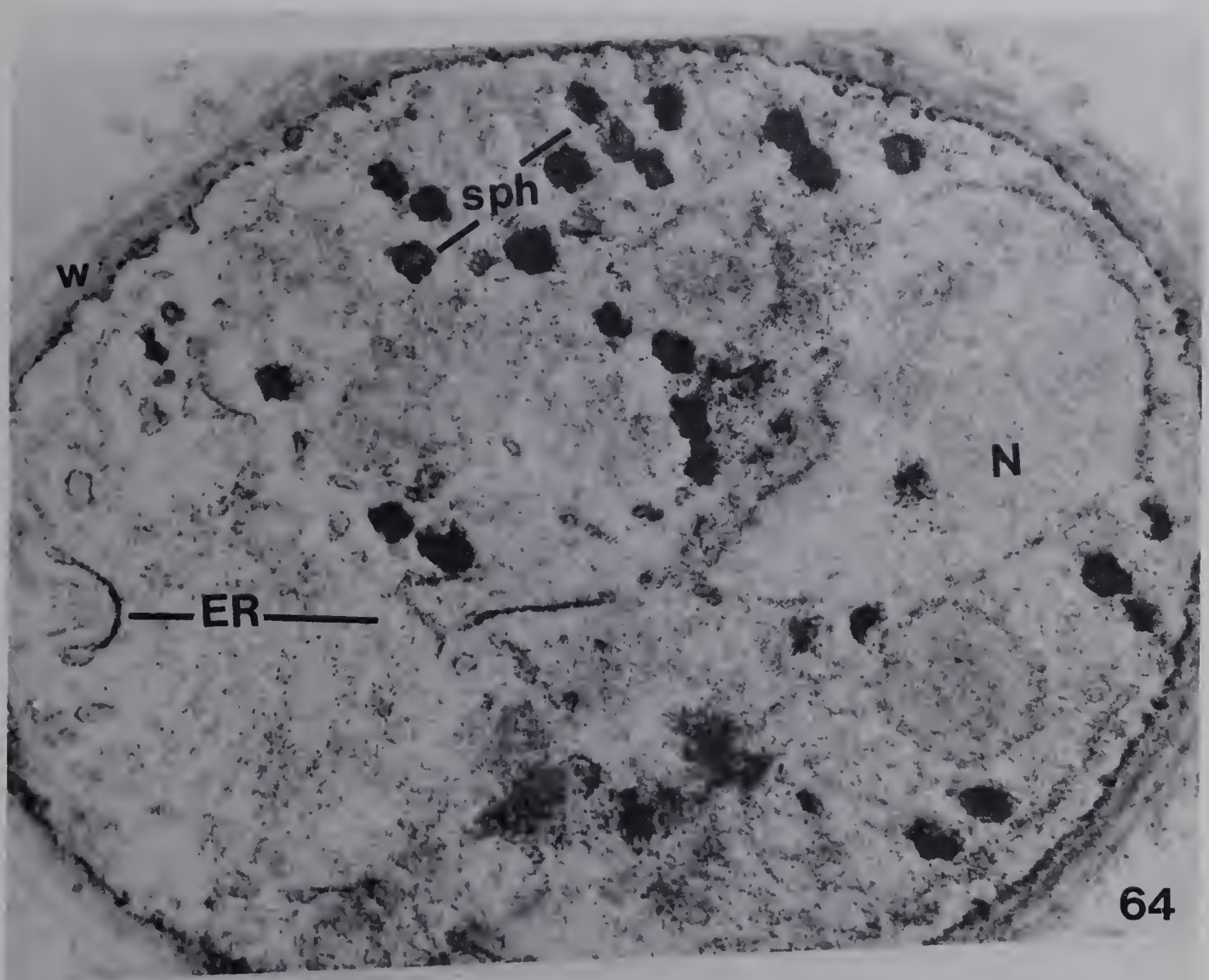
X 52,800



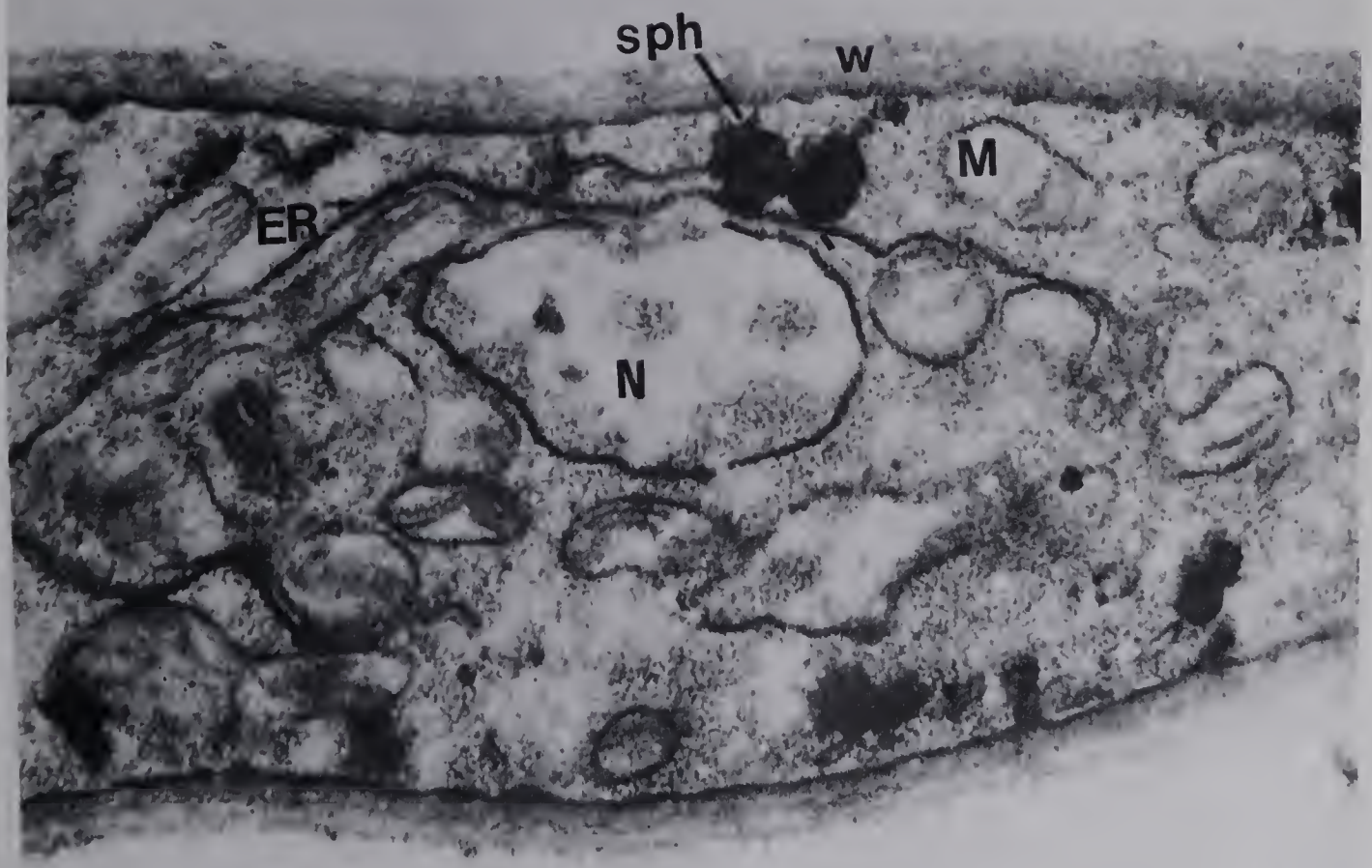
63

Figure 64. Transverse section of a germinated conidium illustrating spherosomes (sph) and endoplasmic reticulum (ER) some of which shows a dilation prior to the formation of spherosomes. $KMnO_4$ - fixed and stained with lead citrate.
X 45,600

Figure 65. A longitudinal section of a germinated conidium of N. crassa showing dense bodies which appear to be delimited by a membrane. These are interpreted as being spherosomes (sph). $KMnO_4$ - fixed, lead citrate-stained.
X 61,600



64



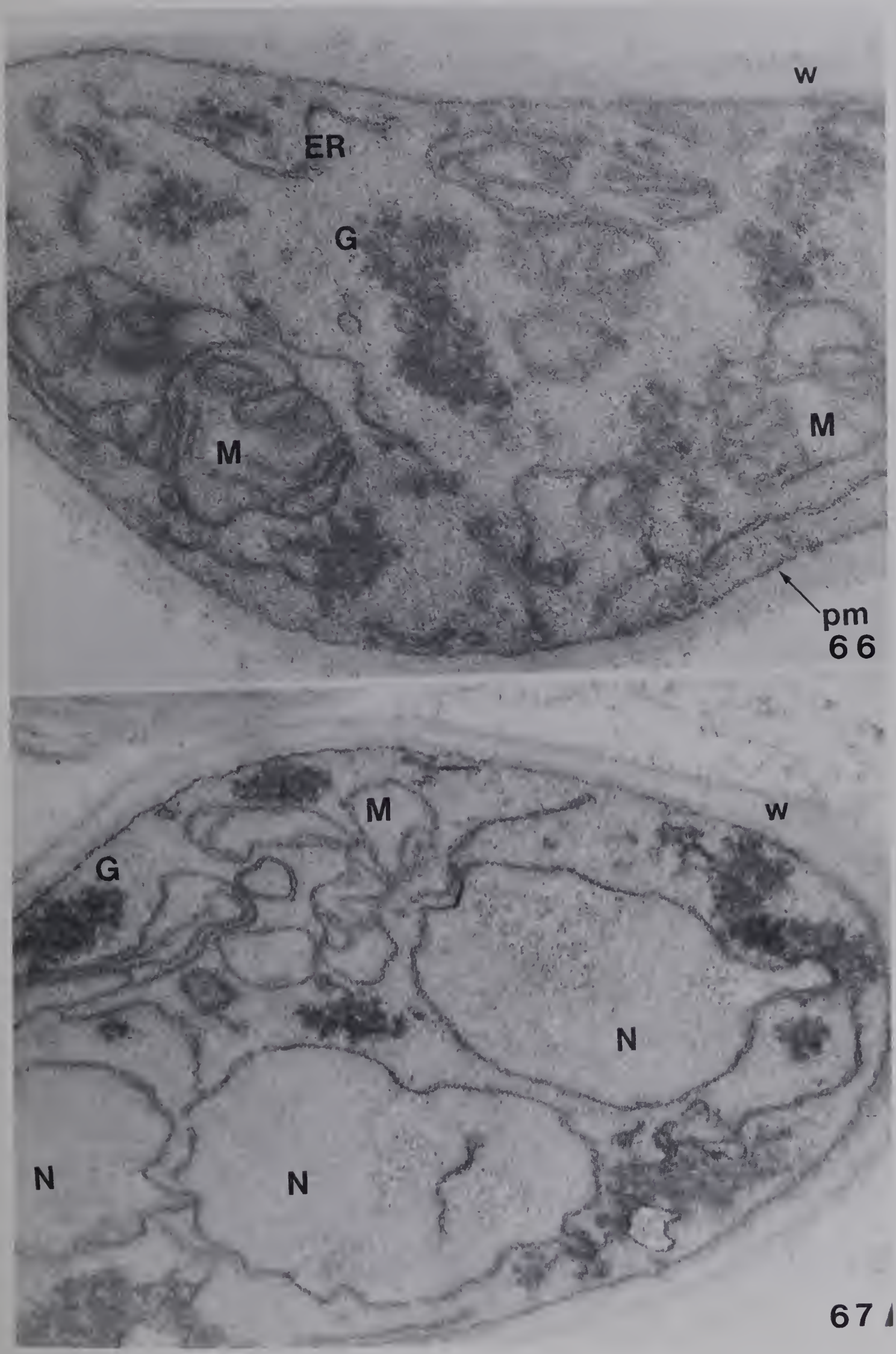
65

Figures 66 and 67. Large aggregations of glycogen in germinating conidium fixed in $KMnO_4$ and stained in lead citrate. It has been suggested that these are a source of energy to the actively metabolizing cell, in addition to supplying short chain carbon units for the synthesis of cytoplasmic constituents.

Figure 66 shows large, swollen mitochondria (M).

Figure 66 X 77,500

Figure 67 X 85,600

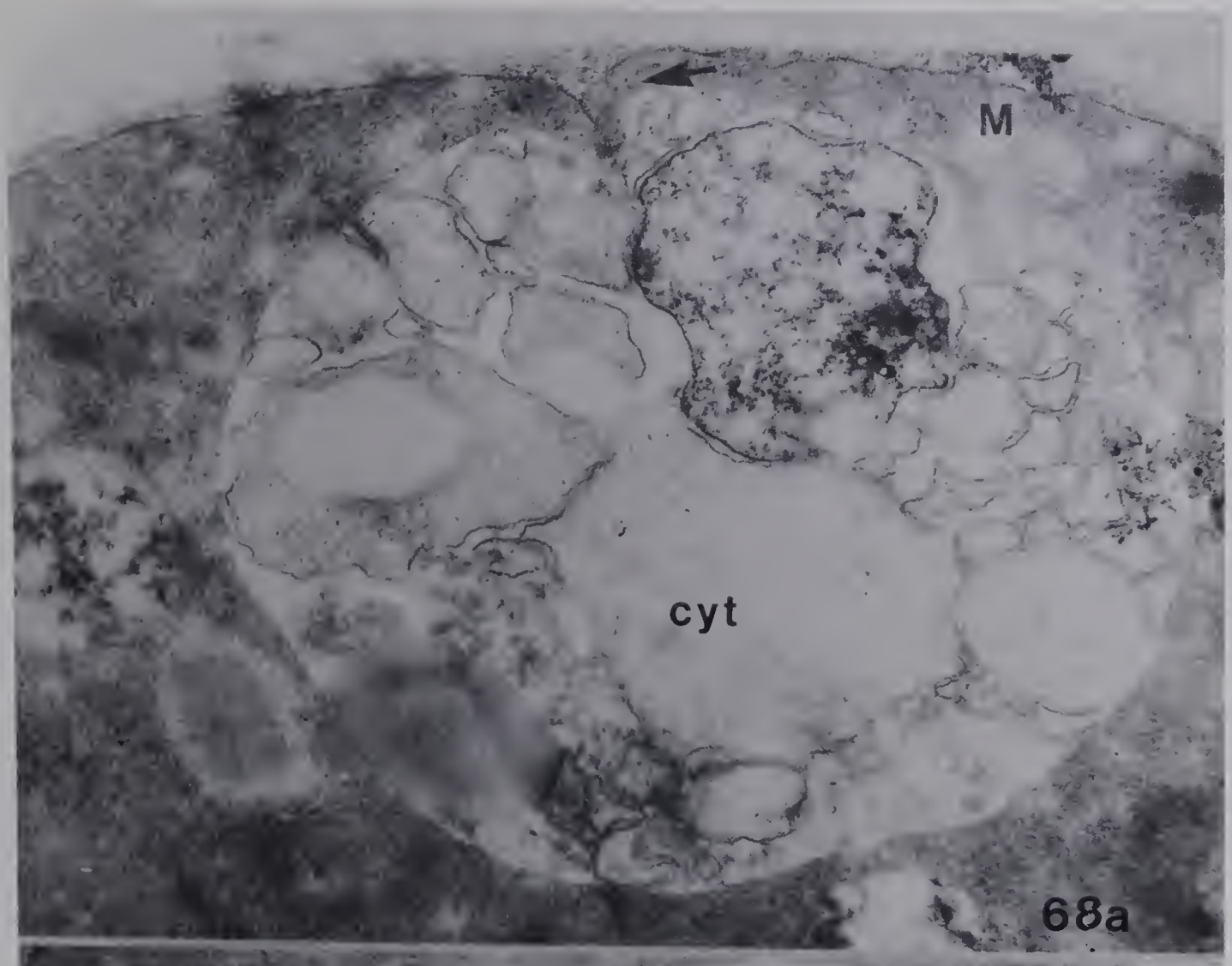


67

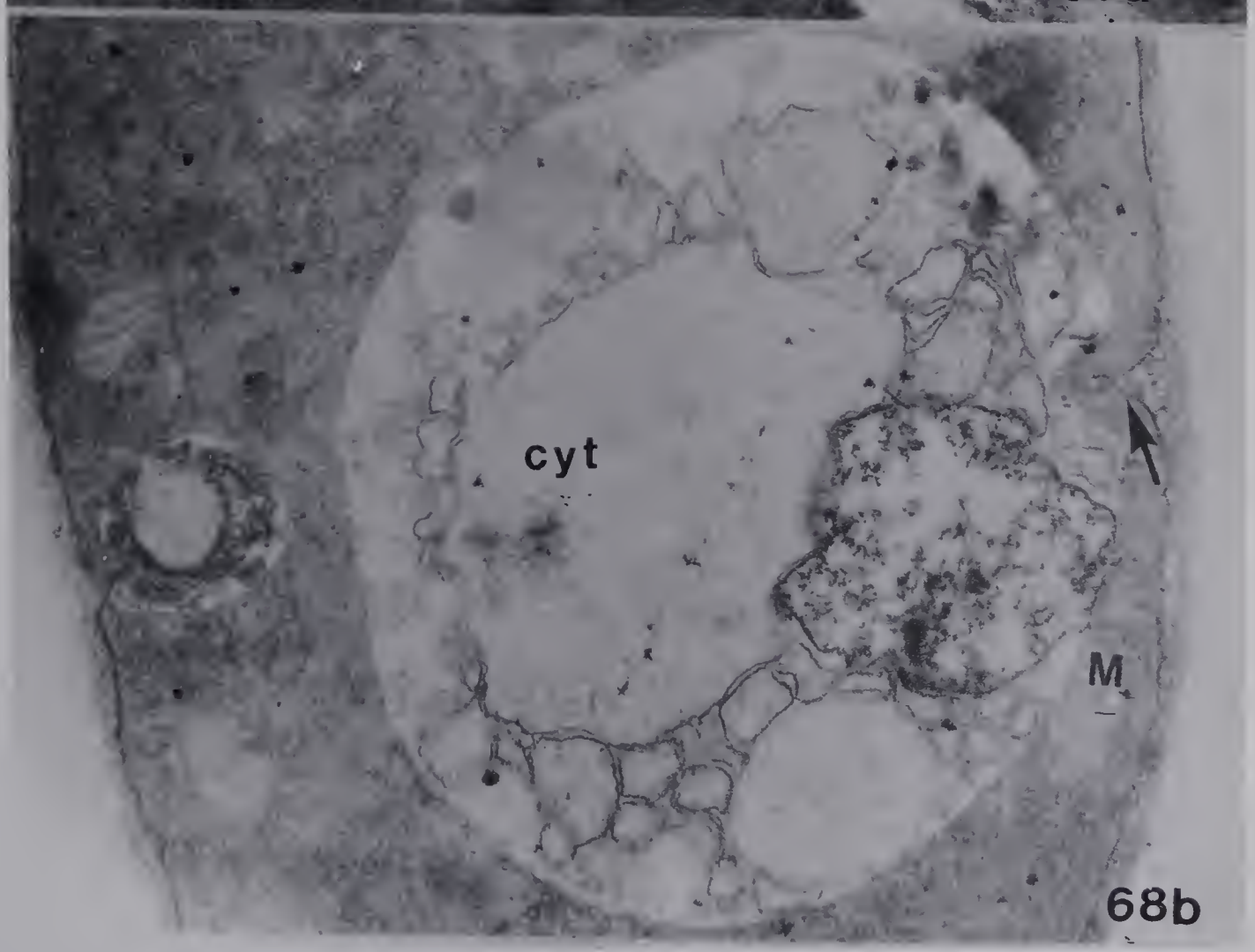
Figures 68 a and 68 b. Longitudinal serial sections of a germinated conidium of N. crassa fixed in glutaraldehyde and osmium tetroxide and stained in uranyl acetate and lead citrate, showing cytolysomes containing various electron opaque structures. Note the invagination of the plasma membrane (arrow).

Figure 68 a X 49,000

Figure 68 b X 46,000



68a

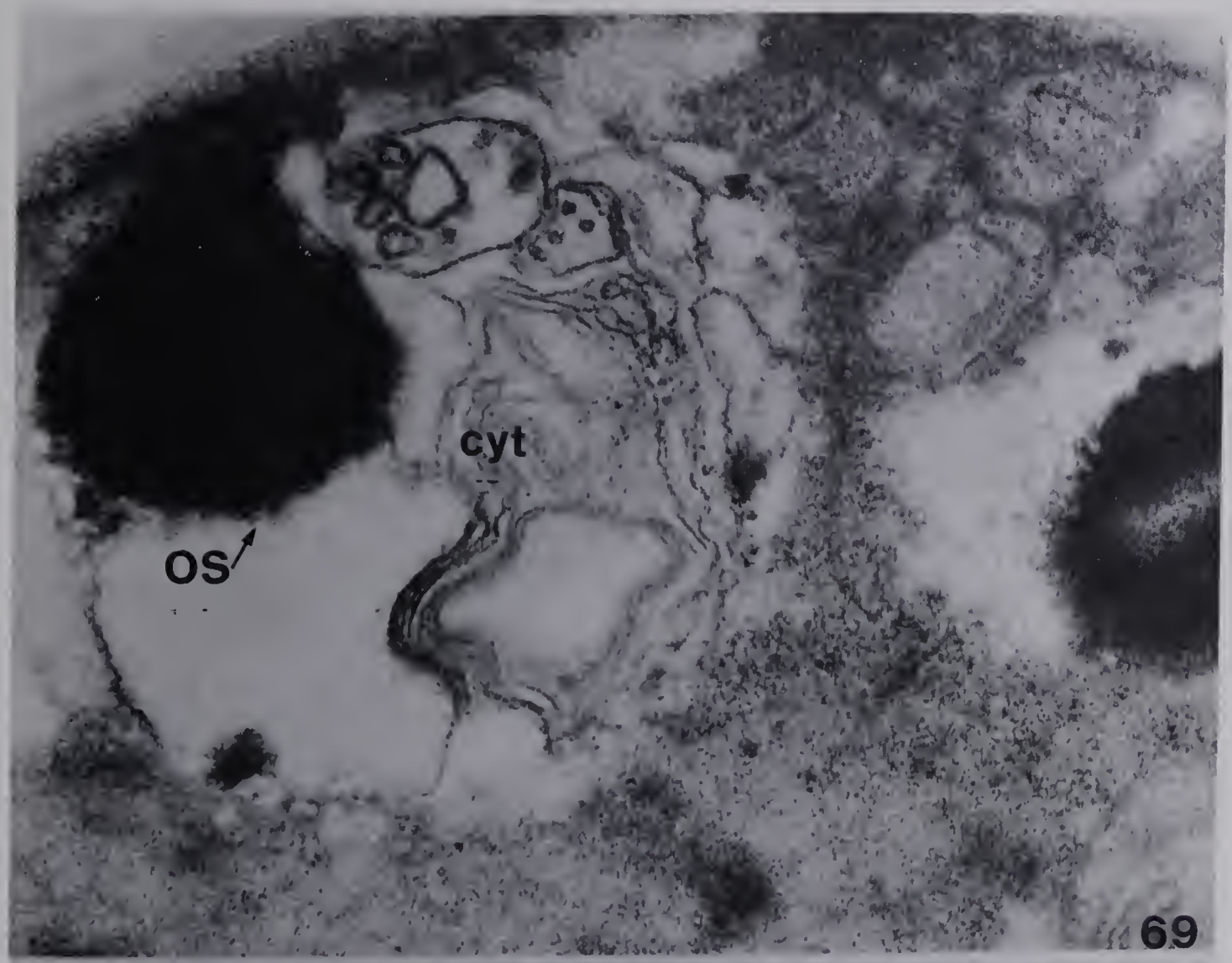


68b

Figures 69 and 70. Enlarged portion of conidia fixed in glutaraldehyde and osmium tetroxide and stained with uranyl acetate and lead citrate showing cytolysomes (cyt) containing osmiophilic bodies, membranous structures and other electron opaque substances.

Figure 69 X 76,000

Figure 70 X 76,500


cyt
OS
69

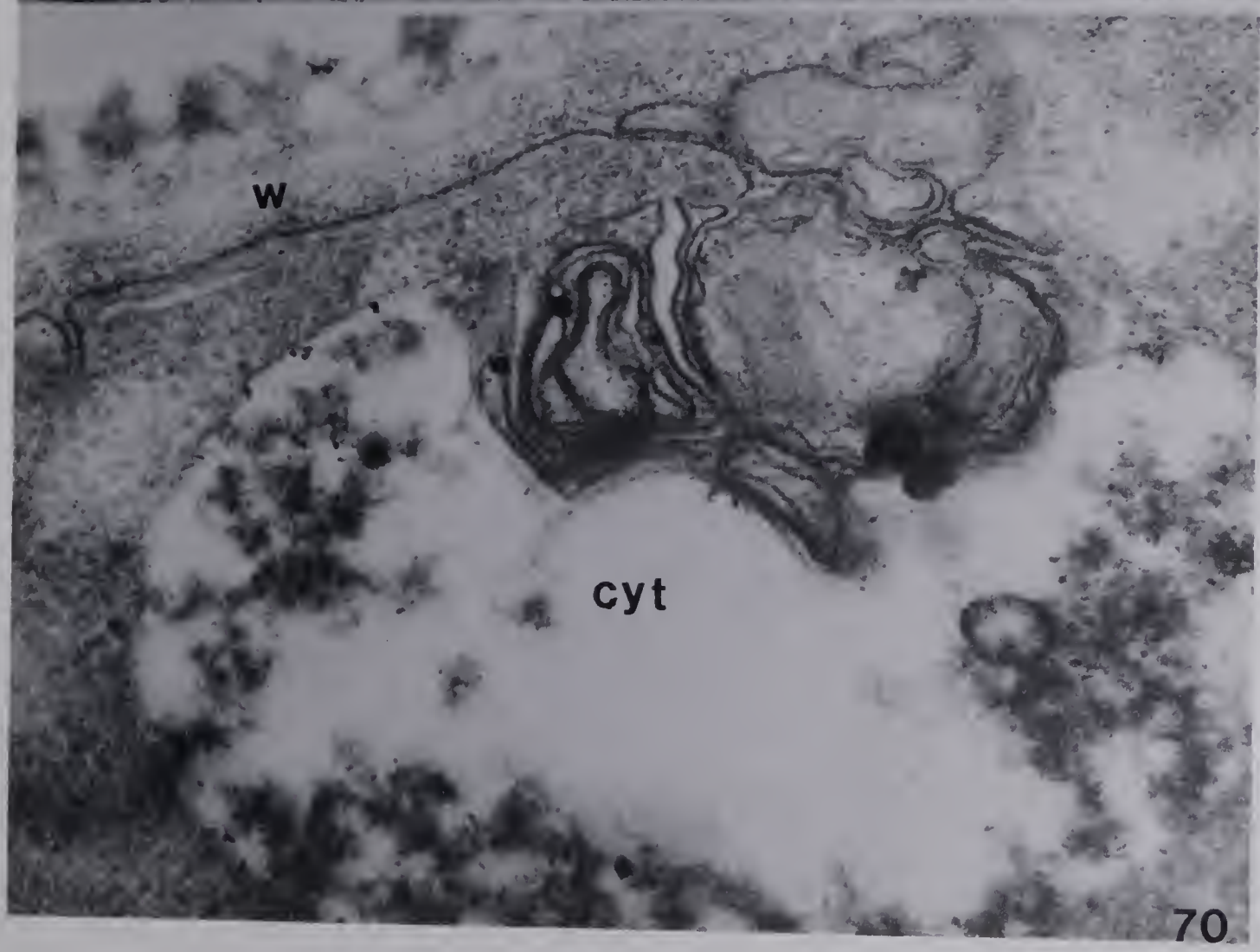
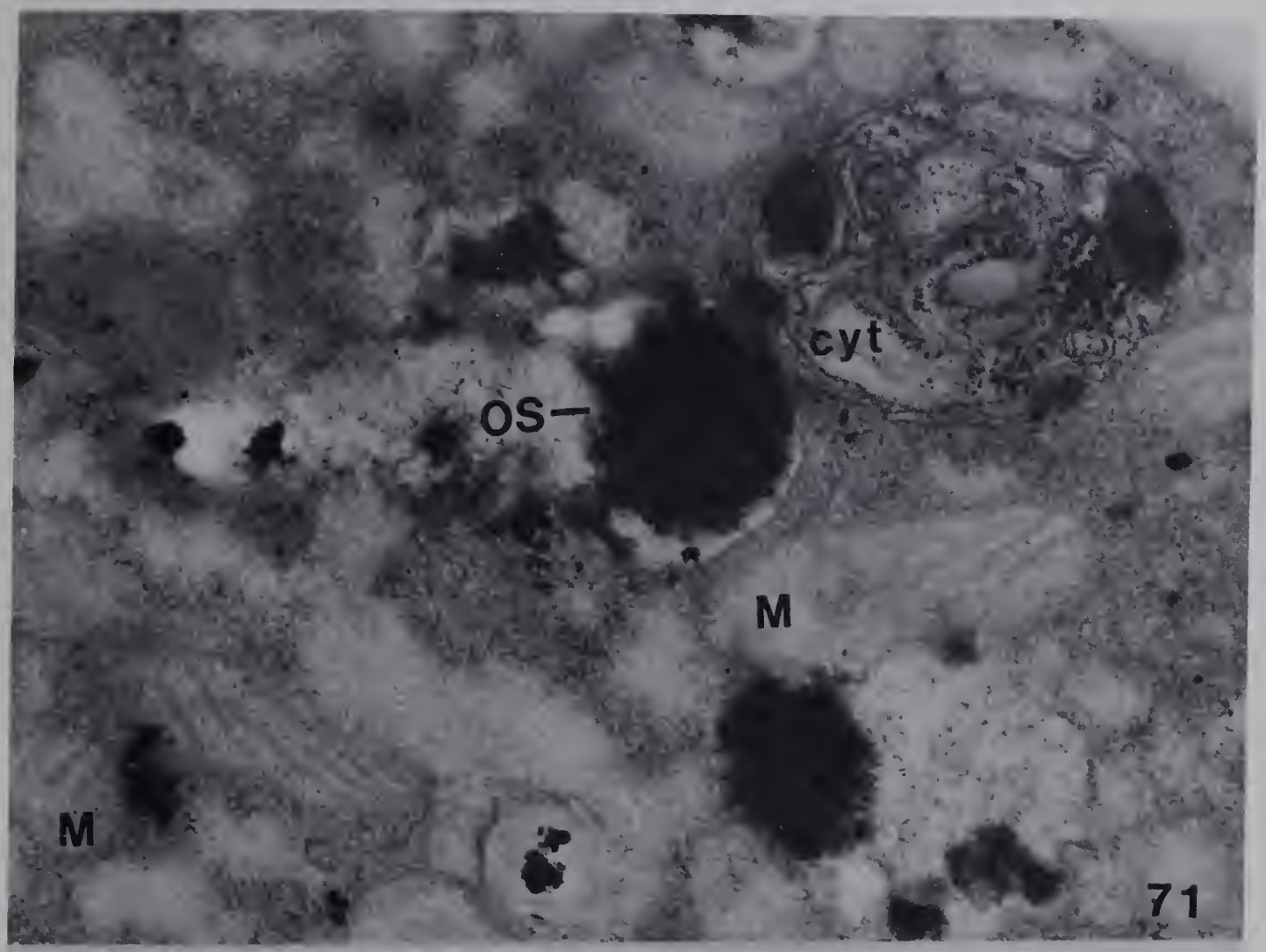

W
cyt
70

Figure 71. An enlarged portion of a glutaraldehyde and osmium tetroxide-fixed conidium stained in uranyl acetate, showing a cytolysome. Also shown are osmiophilic bodies (OS).

X 75,600

Figure 72. Enlarged portion fixed and stained as Figure 71 illustrates a cytolysome containing a captured mitochondrion.

X 65,500


cyt
OS
M
M
71

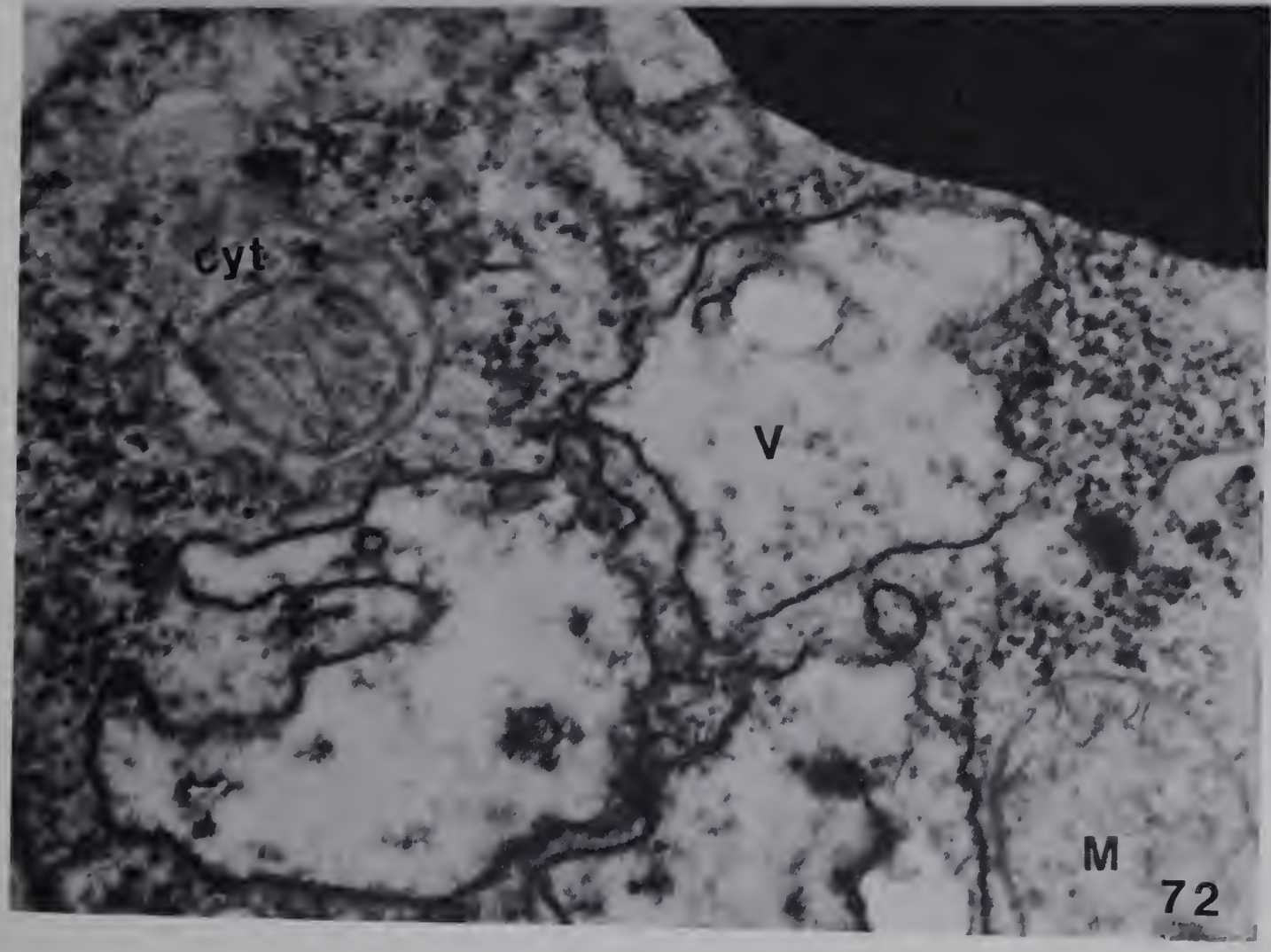

cyt
V
M
72

Figure 73. Portion of an enlarged conidium showing mitochondria (M) glycogen (G) and several cytolysomes containing mesosomes (mes) and osmiophilic bodies in varying stages of degeneration, as well as other unstructured material. Glutaraldehyde and osmium tetroxide fixation. Stained with uranyl acetate and lead citrate.

X 54,000

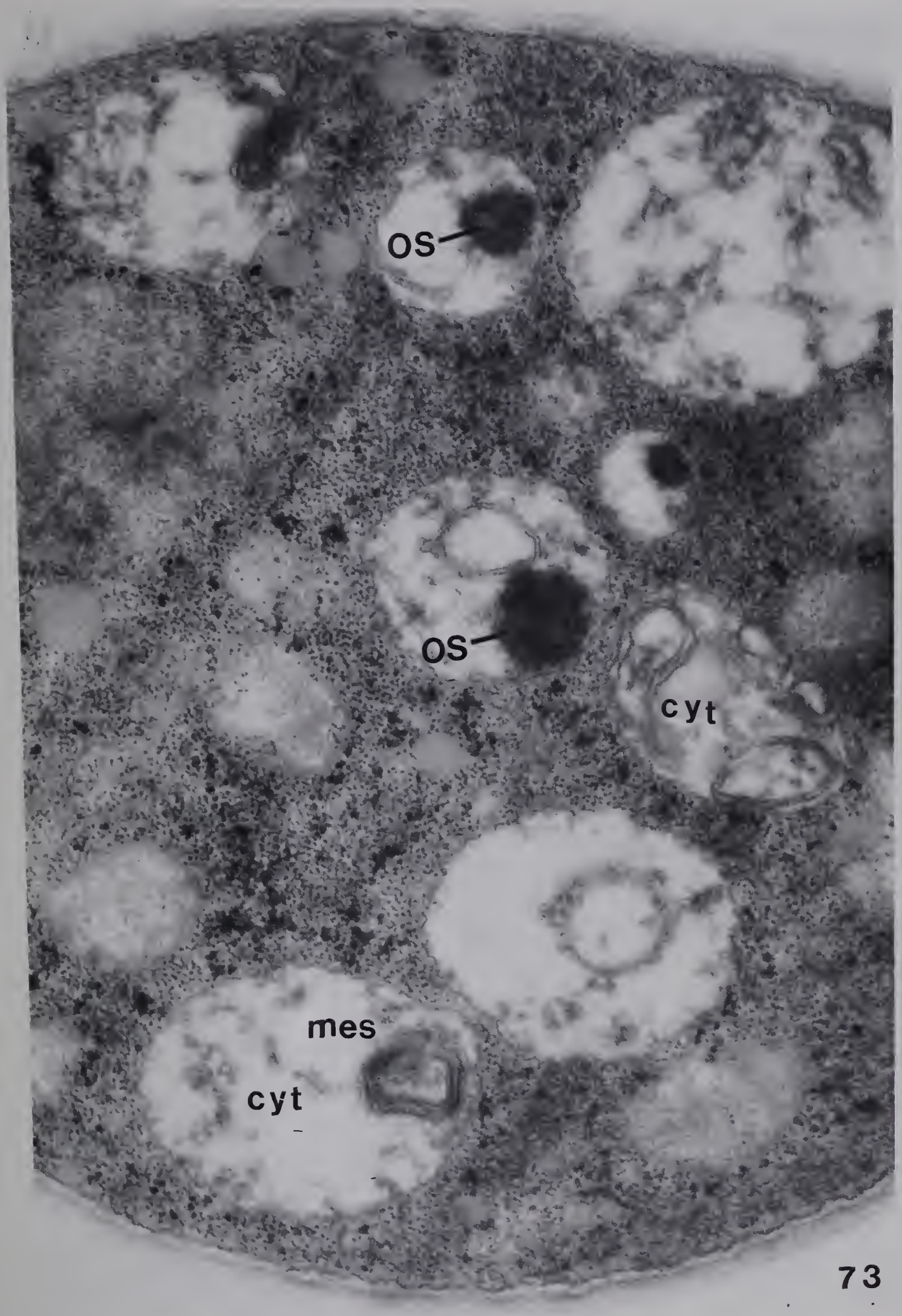
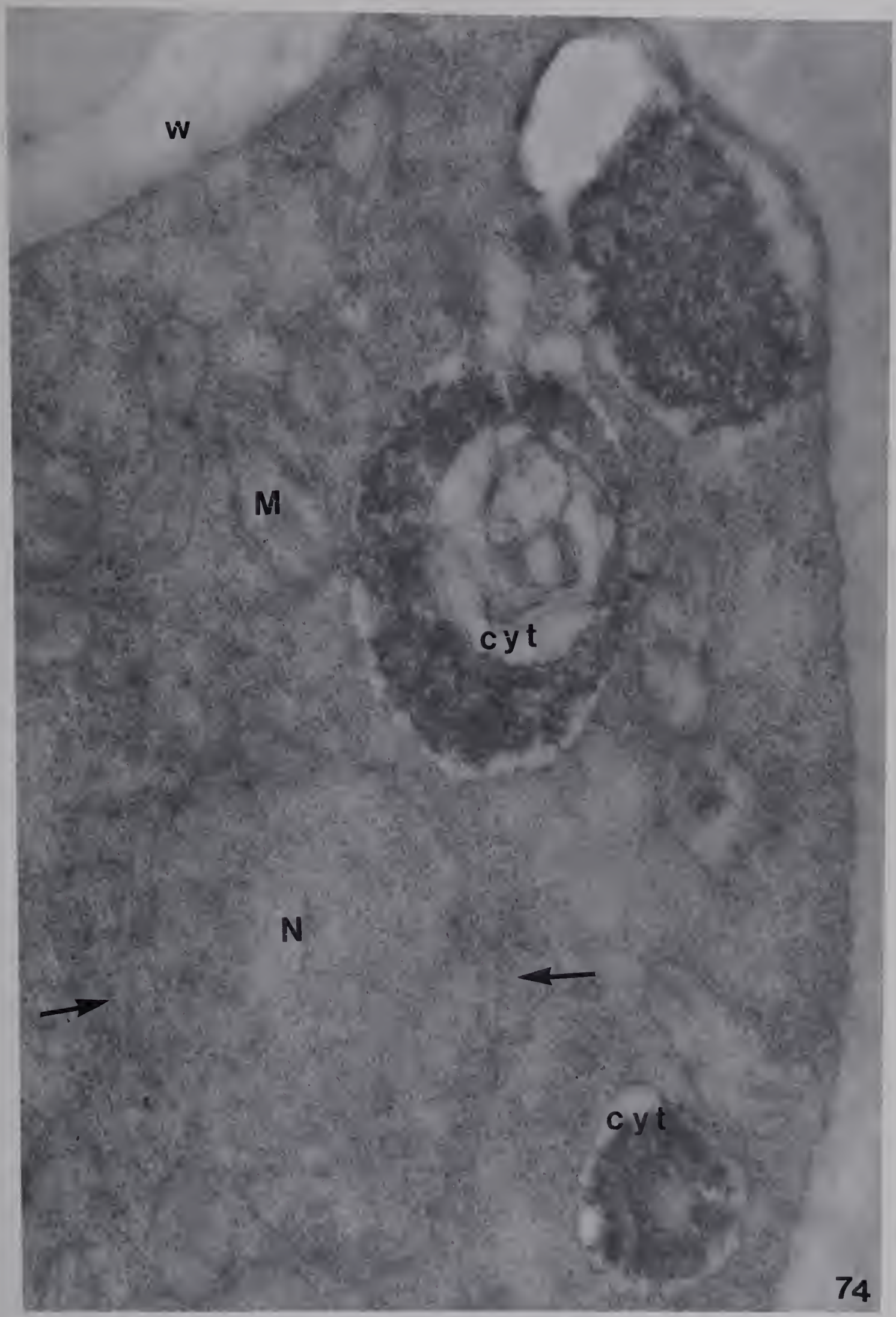

OS
OS
cyt
mes
cyt

Figure 74. An enlarged portion of a germinated conidium of N. crassa fixed in glutaraldehyde and osmium tetroxide and photographed following staining of the section with uranyl acetate and lead citrate. Note the microtubules in the vicinity of the nucleus (N). Also shown are a number of mitochondria (M) and several cytolysomes (cyt), one of which contains particles of high electron opacity as well as membranous structures.

X 51,000

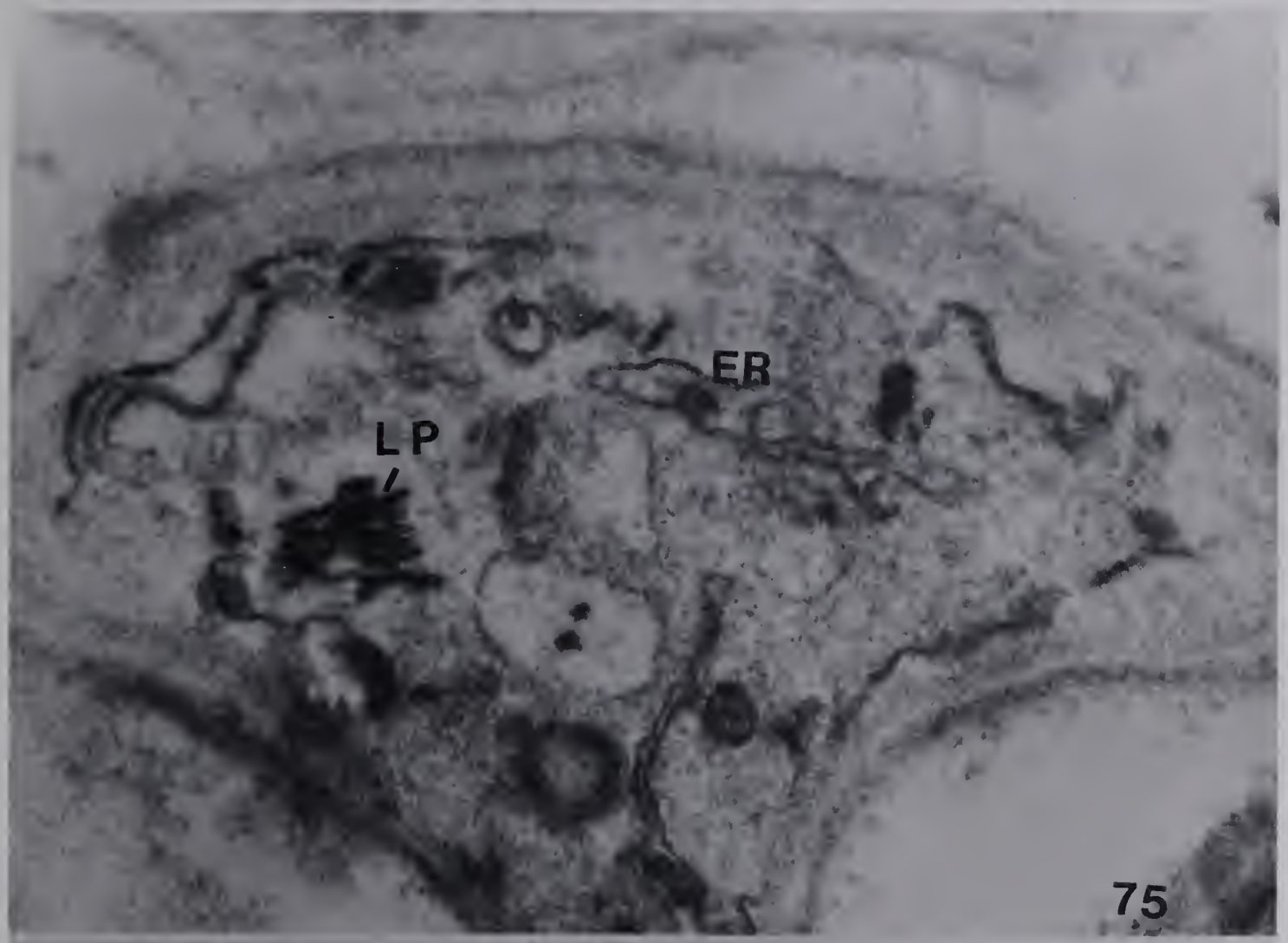

W
M
cyt
N
cyt

Figures 75 and 76. Longitudinal sections of germinated conidium of N. crassa undergoing senescence. Lipoproteinic sheets (LP) are shown. These sheets may be analogous to the thylakoids described by Frey-Wyssling and Schwegler (loc. cit.) and others.

$KMnO_4$ - fixed, lead citrate-stained.

Figure 75 X 83,500

Figure 76 X 90,000


EB
LP
75

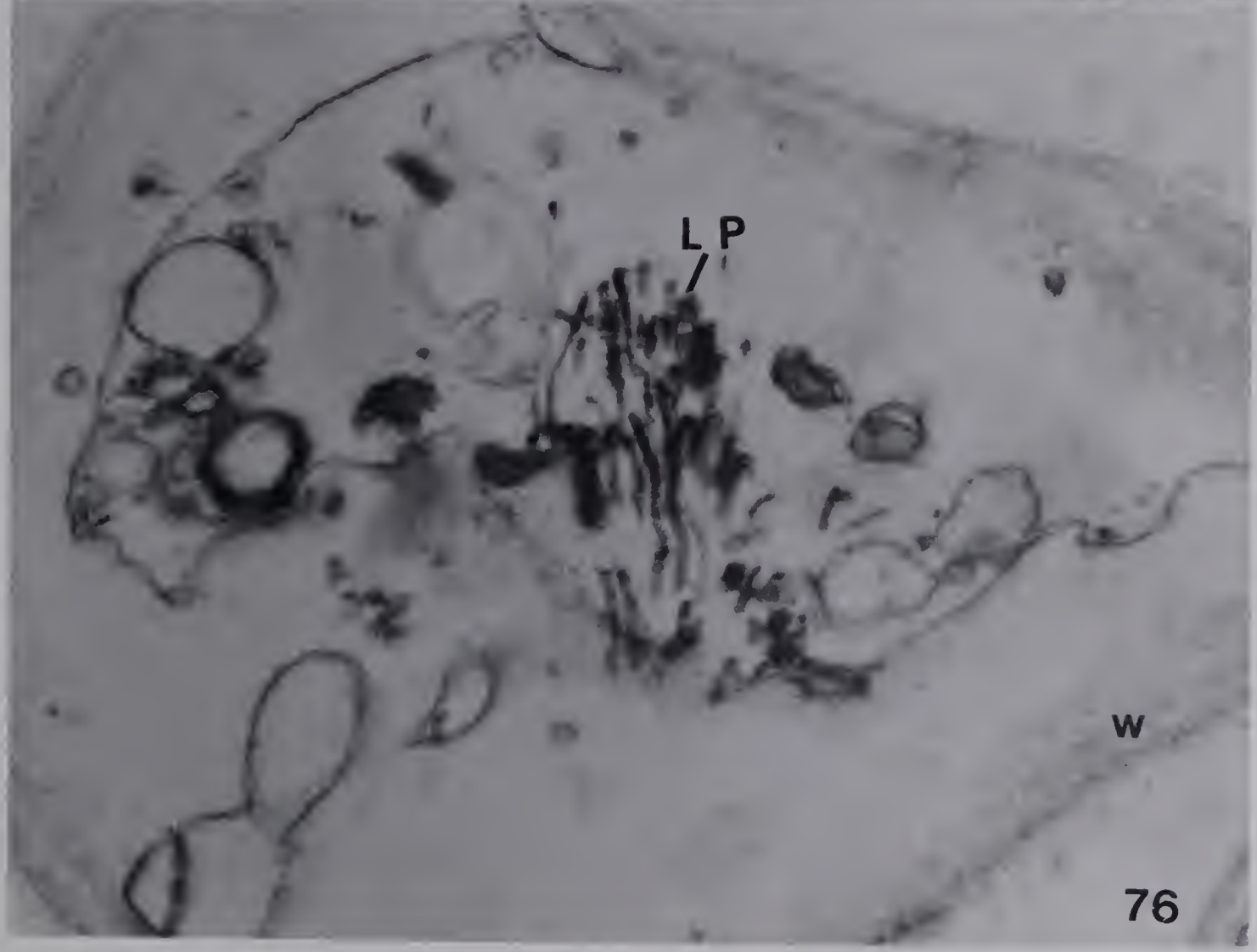
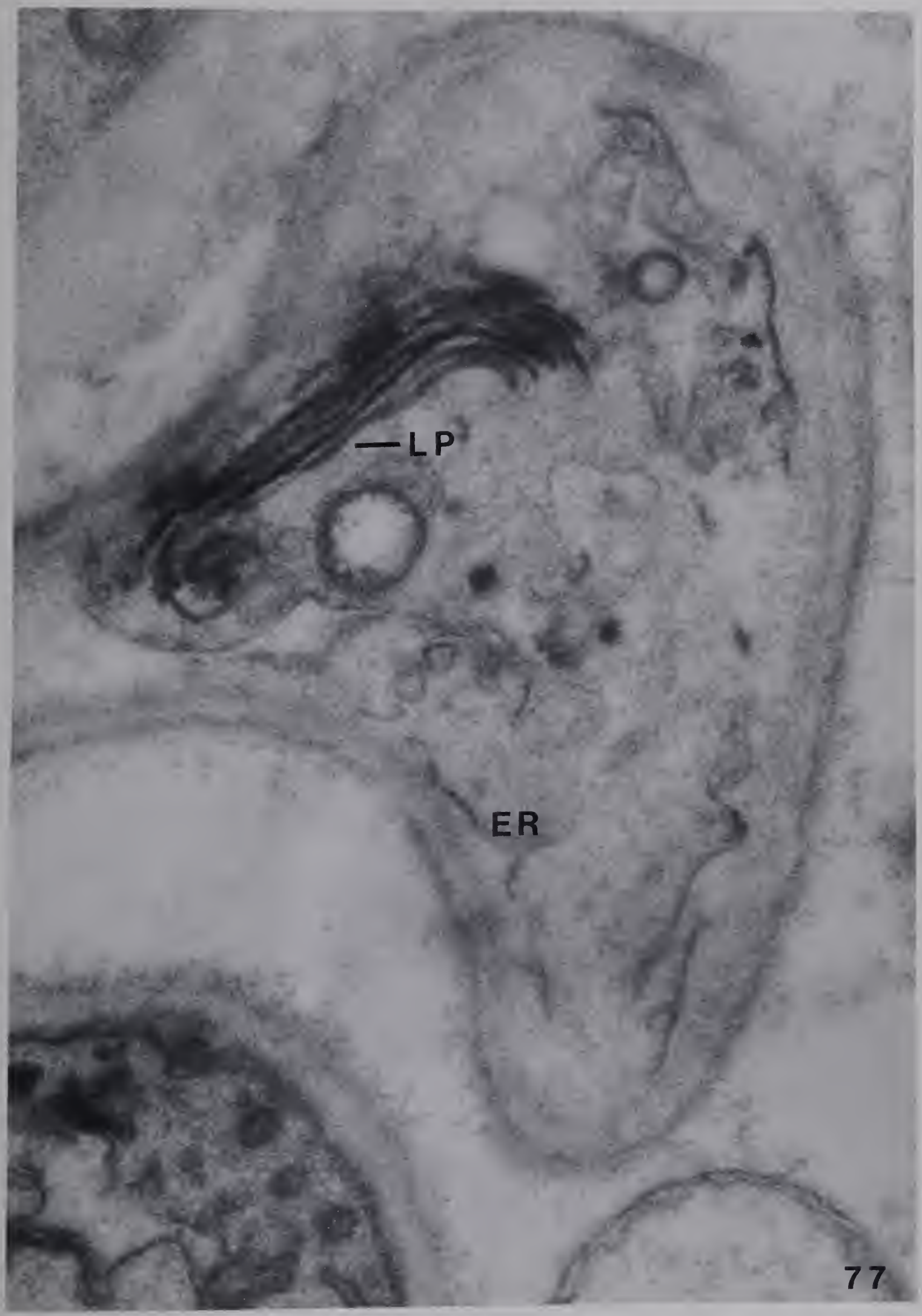

LP
W
76

Figure 77. Micrograph of a longitudinal section of a germinated conidium showing structures marked (LP) which, on the basis of the intense reaction with $KMnO_4$, are interpreted as lipoproteinic sheets.

$KMnO_4$ - fixed lead citrate-stained.

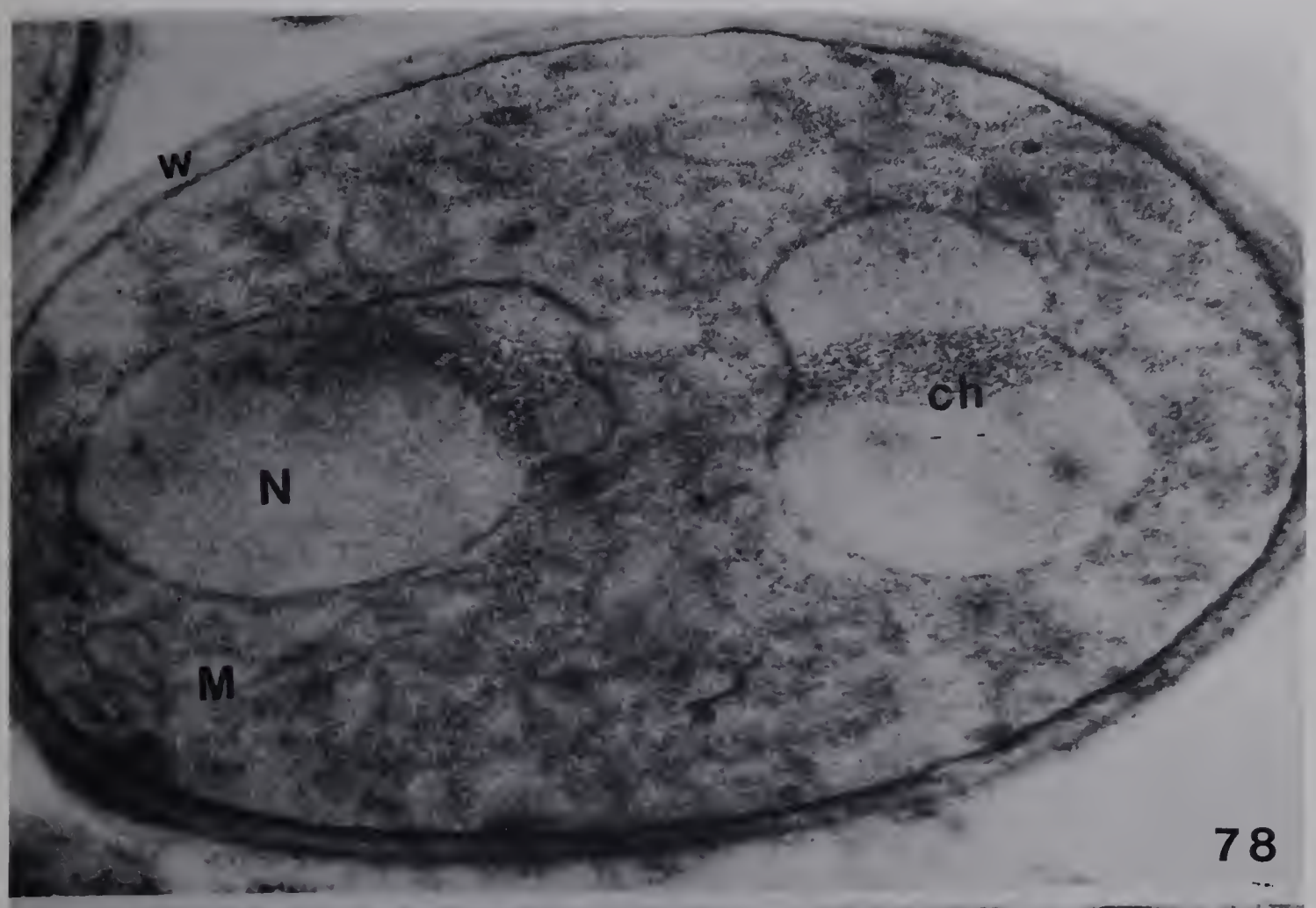
X 90,000


LP
ER

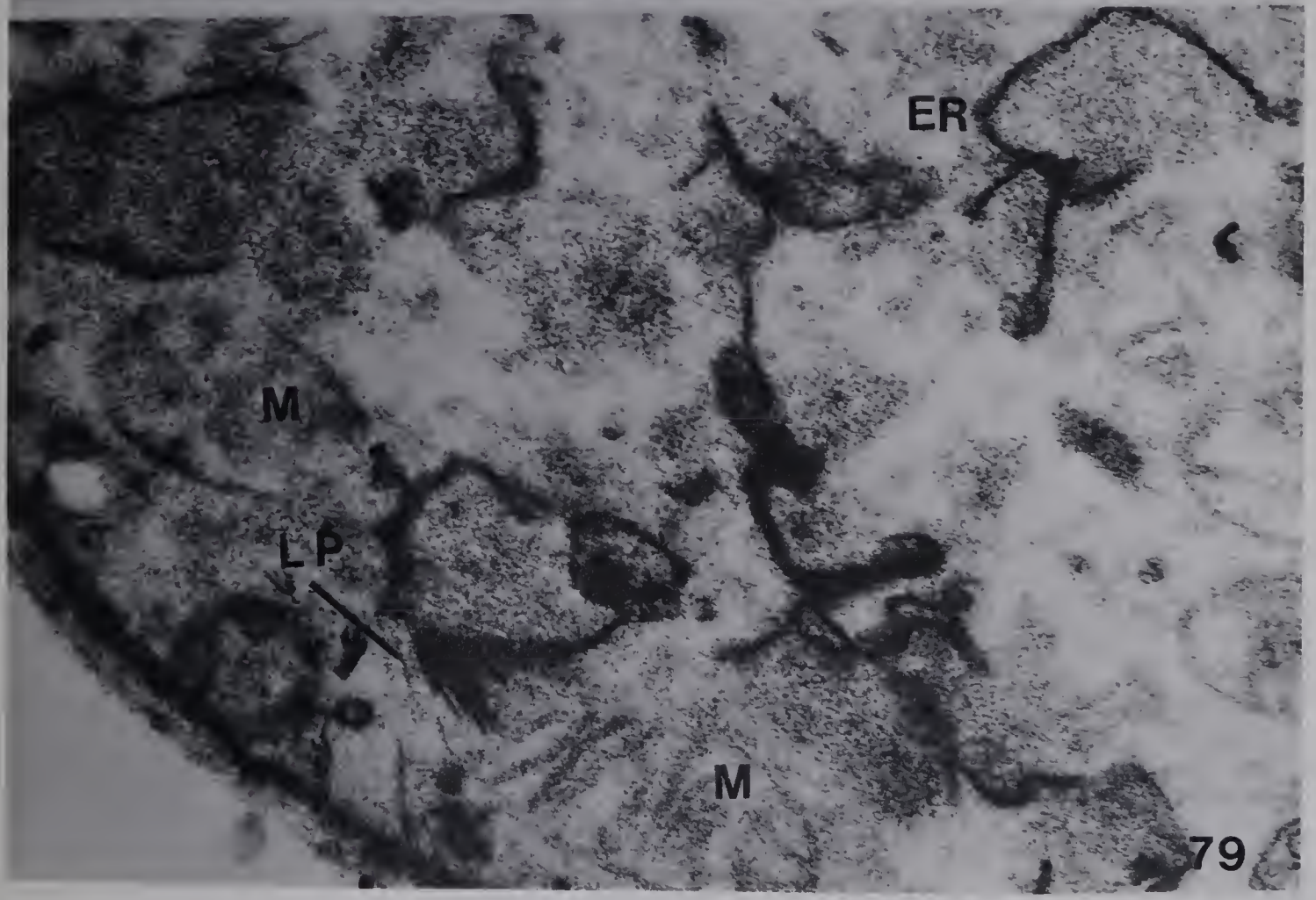
Figures 78 and 79. Electron micrograph of control. Conidia taken from a 10 day old culture served as a control. $KMnO_4$ - fixed, lead citrate-stained.

Figure 78 X 40,800

Figure 79 X 63,800



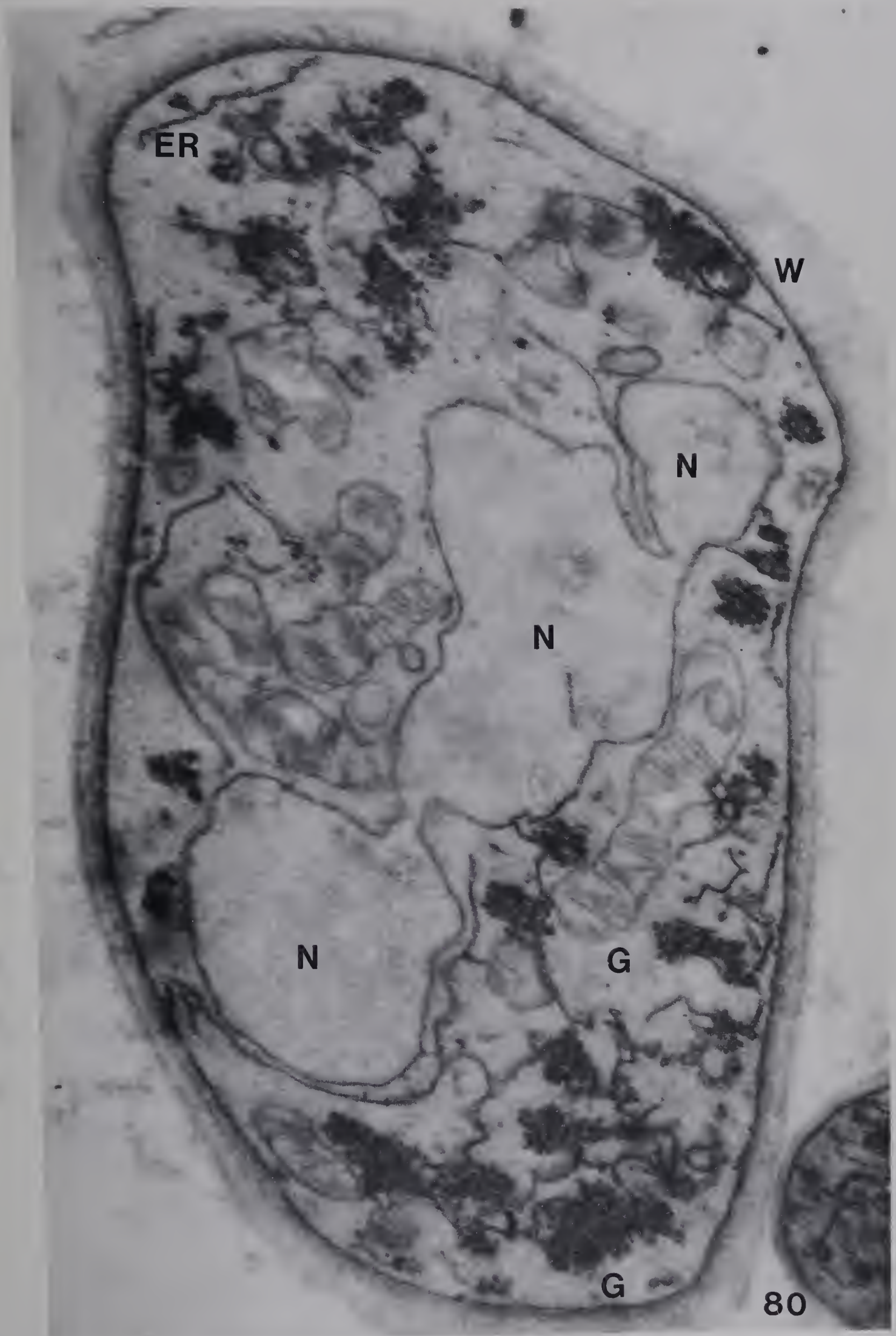
78



79

Figure 80. $KMnO_4$ - fixed germinated conidium of N. crassa stained with lead citrate illustrating nuclei with interconnections. Also seen are glycogen granules (G) and mitochondria (M).

X 82,000



80

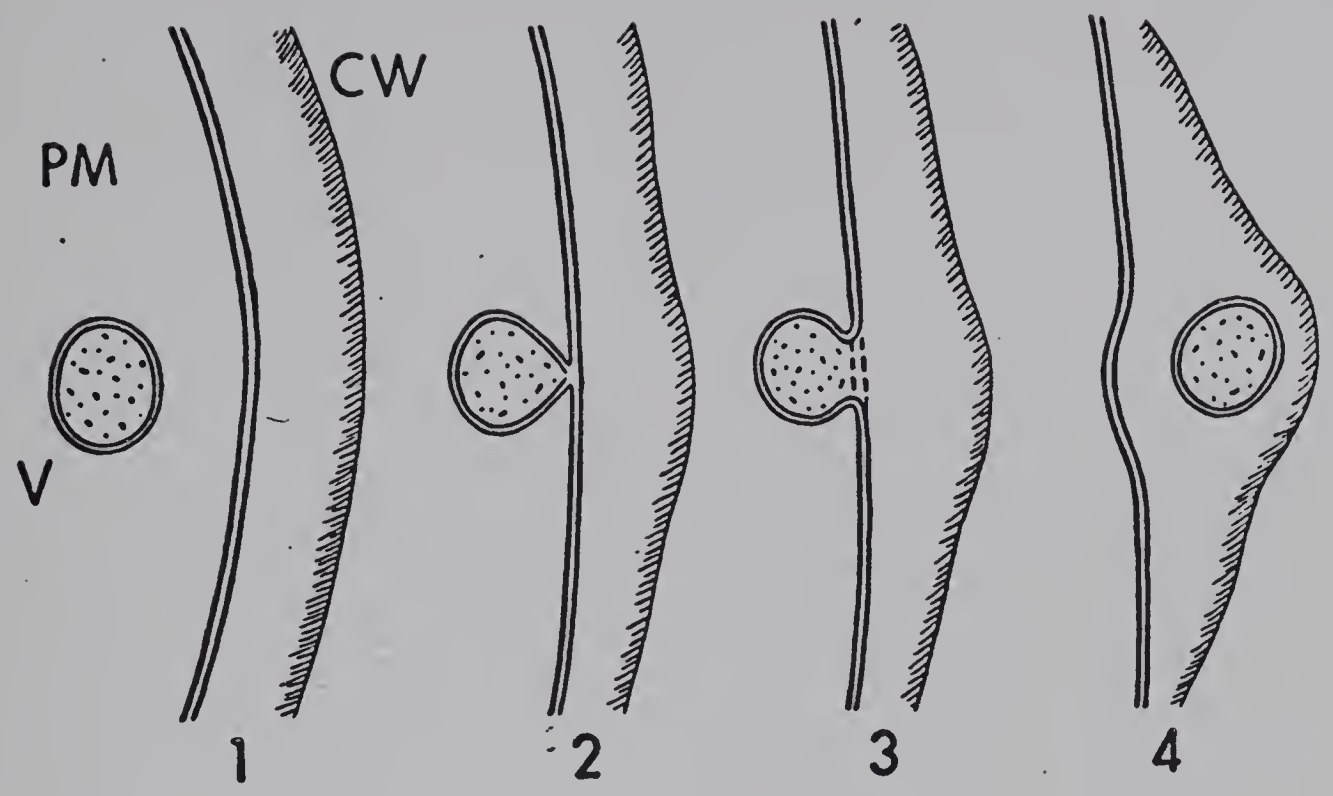


FIGURE 81: SCHEMATIC DIAGRAM OF THE DIFFERENT PHASES IN LOM ASOME DEVELOPMENT BASED ON THE SECRETORY FUNCTION OF THE ORGANELLE (see also Figure 38b)

1. Formation of a vesicle and subsequent movement to periphery of the cell.
2. Contact and fusion of the two membranes.
3. Disintegration of the fusion area.
4. Expulsion stage. Secretory product now lies outside the plasma membrane.

V - vacuole, PM - plasma membrane, CW - cell wall